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COCHLEAR PATHOLOGY IN PRESBYCUSIS

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A survey of the temporal bone collection at the Massachusetts Eye and Ear Infirmary reveals 21 cases that meet the criterion for the clinical diagnosis of presbycusis. It is evident that the previously advanced concept of four predominant pathologic types of presbycusis is valid, these being sensory, neural, strial, and cochlear conductive. An abrupt high-tone loss signals sensory presbycusis, a flat threshold pattern is indicative of strial presbycusis, and loss of word discrimination is characteristic of neural presbycusis. When the increments of threshold loss present a gradually decreasing linear distribution pattern on the audiometric scale and have no pathologic correlate, it is speculated that the hearing loss is caused by alterations in the physical characteristics of the cochlear duct, and the loss is identified as cochlear conductive presbycusis. It is clear that many individual cases do not separate into a specific type but have mixtures of these pathologic types and are termed mixed presbycusis. About 25% of all cases of presbycusis show none of the above characteristics and are classified as indeterminate presbycusis.

KEY WORDS — cochlear pathology, deafness of aging, presbycusis.

INTRODUCTION

Aging cochleae present disorders that are symmetric in paired ears; however, the extent of involvement of the different cytologic structures proceeds on an uneven front. The result is different combinations of deficits in hair cells, the stria vascularis, and neurons that for the individual are genotypic and inherited. The functional expressions of the cochlear disorders present a wide spectrum of abnormal pure tone threshold patterns and diminished word discrimination scores.

In previous reports emanating from this laboratory, it was emphasized that presbycusis could be viewed as occurring in four pathologic types or in combinations thereof.[1-5] Three types are based on visual correlations of audiograms and cytocochleograms; thus, abrupt high-tone threshold losses are attributed to sensory cell loss, flat threshold losses to strial atrophy, and diminished word discrimination to loss of cochlear neurons. Because no pathologic correlate is evident for the gradual descending audiometric pattern, it is reasoned that a fourth type of presbycusis is caused by an inner ear conductive disorder that has no pathologic correlate on light microscopy.

We have examined a larger number of cases of presbycusis that reside in the temporal bone collection at the Massachusetts Eye and Ear Infirmary and have correlated the audiometric test data with quantitative assessments of cochlear disorders. The results of these studies show that the concept of four predominant pathologic types is valid; however, it is clear that many cases show mixtures of these types, and furthermore, in about 25% of cases the cochlear changes on light microscopic examination are not adequate to explain the hearing losses.

MATERIALS AND METHODS

A survey of the entire collection of 1,500 serially sectioned temporal bones reveals 21 cases that meet the criteria of having sensorineural hearing losses characterized by insidious onset, bilateral symmetry, and progression into old age without any clinical evidence of other ear disorders. The age, sex, occupation, test to autopsy interval, and cause of death for each case is presented in Table 1. The number of cases is too small for statistical analysis; therefore, visual correlations of the audiometric data and cytocochleograms form the basis for our judgments. The pairs of cochleae show similar pathologic changes; therefore, the cochlea of each pair judged to have the best histologic preservation and preparation has been chosen for quantitative cytologic study.

The 8-kHz frequency was uniformly poorly heard in all of these aged individuals. The thresholds for this frequency ranged from 60 dB to 100 dB for an average of 75.8 in 17 cases. In 4 cases, the tone was not heard at maximum stimulation intensities. For this reason 8 kHz does not play a significant role in our differentiation of different pathologic types of presbycusis. A casual inspection of the audiograms and related cytocochleograms shows that a loss of

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TABLE 1. PATIENT DATA

Type of Presbycusis	*Case*	*Age*	*Sex*	*Occupation*	*TAI*	*Cause of Death*
Sensory	1	93	M	Shoemaker	10 y	Cardiac failure
Neural	2	71	M	Shipyard carpenter	16 y	Gastric cancer
	3	81	M	Factory worker	13 y	Cardiac failure
	4	91	F	Unknown	7 mo	Bronchopneumonia
Strial	5	76	F	Homemaker	3 y	Hepatic cancer
	6	72	F	File clerk	4 y	Myocardial infarct
	7	72	M	Military service	3 y	Cerebral hemorrhage
	8	73	F	Homemaker	5 y	Gastric hemorrhage
Cochlear conductive (hypothetical)	9	91	M	Unknown	6 mo	Pulmonary embolus
	10	90	F	Store manager	8 mo	Myocardial infarct
	11	87	M	Prize fighter, cook	1 yr	Pulmonary cancer
Mixed	12	89	F	Seamstress	2 mo	Cardiac failure
	13	81	F	Musician	18 mo	Myocardial infarct
	14	82	M	Construction riveter	11 y	Leukemia
	15	85	F	Unknown	11 mo	Cardiac failure
	16	96	F	Factory seamstress	4 y	Cardiac failure
Indeterminate	17	89	M	Factory worker	16 y	Pulmonary cancer
	18	85	F	Stockbroker	5 y	Cardiac failure
	19	75	F	Homemaker	1 mo	Pharyngeal cancer
	20	84	M	Attorney	2 y	Myocardial infarct
	21	67	F	Homemaker	6 mo	Colonic cancer

TAI — test to autopsy interval.

cochlear neurons shows little or no quantitative relationship to elevation of thresholds for pure tones, but is expressed audiometrically in terms of diminished word discrimination scores.

All of the temporal bones had been prepared by the standard method of being decalcified, embedded in celloidin, and serially sectioned in the horizontal plane at a thickness of 20 µm. Every 10th section was stained with hematoxylin and eosin and mounted on a glass slide. The cochleae were graphically reconstructed according to the method of Guild[6] and Schuknecht.[7,8] Inner and outer hair cells were plotted separately as being present or absent. The neuronal populations were counted with the aid of an ocular grid and multiplied by 10. A correction factor of 0.9 was used to compensate for neurons located at the interface of sections, where they would be subject to double counting. The method of Otte et al[9,10] was used to display the neuronal data. In this method a vertical line is drawn through the neuronal spiral reconstruction to divide the spiral ganglion into four segments, and the total count for each segment is determined. For a 32-mm-long cochlea, these segments provide innervation for the organ of Corti approximately as follows: segment 1, 0 to 6 mm; segment 2, 6 to 15 mm; segment 3, 15 to 22 mm; and segment 4, 22 to 32 mm. Segmentalizing the neuronal counts minimizes the inaccuracies of attempting to spatially correlate the spiral ganglion with the organ of Corti on a more precise basis. The stria vascularis was plotted in terms of estimated loss of volume of strial tissue, with no attempt to differentiate the three cell types (marginal, intermediate, basal) that constitute this organ.

The data on the hair cells, neurons, and the stria vascularis were transferred to histograms in which black filling indicated the percent loss as a function of distance along the cochlear duct as measured from the basal end. The total loss of strial tissue for each cochlea was determined by measuring the percent of black filling in the histogram with an image analysis computer system (Zeiss Videoplan I, CPU-Z80). The neuronal populations were plotted as losses compared to the mean for neonatal cochleae. The length of the cytocochleogram was expanded or contracted overall, if necessary, to fit our 32-mm graphic format. The 21 cochleae undergoing study varied in length from 26.7 to 36 mm, for an average of 32.2 mm. The audiogram for each case was placed on a parallel coordinate of equal length to the cytocochleogram, with frequency located on a data-based anatomic frequency scale. The final product for each cochlea is a cochlear chart consisting of matching audiogram and cytocochleogram.

RESULTS

We established a set of criteria for significant pathologic change based on factors that we believe are reasonable but admittedly somewhat arbitrary. The criterion for sensory presbycusis is the presence

TABLE 2. FINDINGS USED FOR CLASSIFICATION

							Neuronal Evaluations as % Less or (More) Than Normal Means for	
Type of Presbycusis	*Case*	*Age*	*Sex*	*Sensory Lesion (mm)*	*Strial Loss (%)*	*Neuronal Count*	*Birth*	*Same Age*
Sensory	1	93	M	11.5*	28.9	18,315	48	(10)
Neural	2	71	M	2.5	0	3,924	89*	81
	3	81	M	3	0.5	12,645	64*	32
	4	91	F	4	9.2	6,111	83*	63
Strial	5	76	F	4	77.1*	18,126	49	13
	6	72	F	4	51.2*	26,667	25	(28)
	7	72	M	1.2	44*	24,903	30	(19)
	8	73	F	0	35.4*	21,891	38	(5)
Cochlear conductive (hypothetical)	9	91	M	1	24.9	23,526	34	(42)
	10	90	F	3	23.2	20,493	42	(10)
	11	87	M	6.5	6.3	21,071	41	(13)
Mixed	12	89	F	3.5	40.3*	15,745	56*	16
	13	81	F	1	42.9*	9,765	73*	48
	14	82	M	12*	7.9	12,429	65*	34
	15	85	F	12*	33.1*	15,552	56*	17
	16	96	F	12*	31.2*	16,173	55*	3
Indeterminate	17	89	M	4	20.7	18,225	49	3
	18	85	F	5	12.4	19,044	46	(2)
	19	75	F	4	15.6	25,704	28	(24)
	20	84	M	2.5	18	23,121	35	(24)
	21	67	F	2.1	17.9	17,937	49	(12)

***Meeting our arbitrary criteria for significant pathologic change.**

of any total loss of hair cells beginning at the basal end of the cochlea that is at least 10 mm in length so as to encroach on the speech frequency area of the cochlea. For neuronal presbycusis the criterion is a loss of 50% or more of cochlear neurons compared to the mean for neonates (35,500). This figure receives some support from the studies of Otte[9] and Pauler et al,[11] in which word discrimination appeared to be diminished with neuronal counts in the range of 15,000 to 20,000. The criterion for strial presbycusis is a loss of 30% or more from the normal volume of strial tissue. This figure is based on the findings of Pauler et al,[12] who showed that hearing losses occurred when there were strial losses of about 30% or greater.

Cochlear conductive presbycusis required both functional and pathologic criteria. The functional criterion is an arbitrary requirement that the audiogram show a linear gradually descending slope over at least a five-octave range, with a difference of at least 50 dB between the best and poorest thresholds and no more than a 25-dB difference between any two adjacent octaves. The pathologic criterion is a requirement that the cochleae must not meet any of the criteria set for significant pathologic change in the sensory cells, neurons, or stria vascularis as described above.

When the cases met the criteria for significant pathologic change in more than one structure, they were classified as mixed presbycusis. Those cases in which cochlear changes did not reach significant levels in any structure, and in which the audiometric thresholds did not meet the criteria for the gradual descending audiogram of cochlear conductive presbycusis, were classified as indeterminate presbycusis.

The quantification of the pathologic changes provides numeric values that are used to classify the 21 cases into one of the six types of presbycusis. The data are presented in Table 2.

Sensory Presbycusis. Sensory cell losses at the extreme basal end of the cochlea are a common occurrence in the aging cochlea; however, the area of involvement rarely extends far enough from the basal end to reach the speech frequency area of the cochlea. Many cochleae of aging persons demonstrate an island of hair cell loss in the 8- to 12-mm region, which tonotopically serves the 4-kHz frequency. We attribute this lesion to acoustic trauma. In cases of severe acoustic trauma the lesion can spread basalward to merge with the sensory presbycusic lesion. In such cases it is not possible to determine to what extent presbycusis and acoustic trauma have played independent causal roles, because the pathologic features

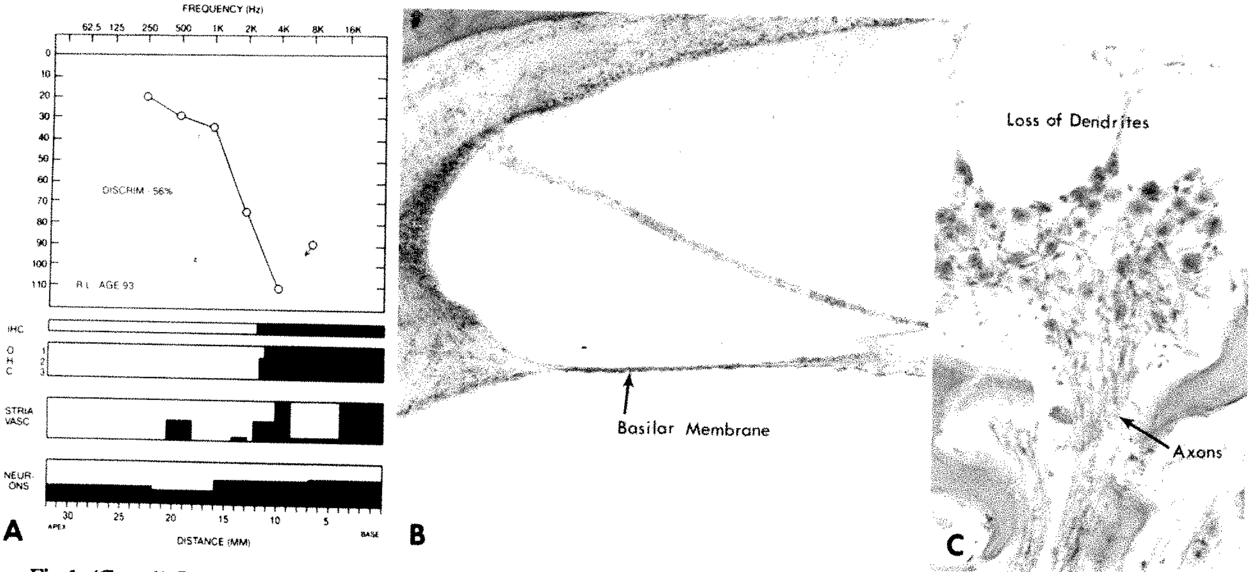


Fig 1. (Case 1) Sensory presbycusis in 93-year-old man. A) Sensory lesion is 12 mm in length. Losses of neurons (48%) and stria (28.9%) do not meet our levels of pathologic significance. B) This view in 8-mm region shows total atrophy of organ of Corti (x57). C) There is retrograde neuronal degeneration in basal turn; note loss of dendritic fibers (x240).

of each are similar. In this case the otologic history may be a more reliable determinant of causation.

The earliest changes, such as a loss of stereocilia, are detectable only by electron microscopy.[13] This is followed by slight distortion and flattening of the organ of Corti and then by a loss of supporting and sensory cells. Eventually the organ of Corti appears as an undifferentiated epithelial mound on the basilar membrane, and it may disappear completely from the basal end of the cochlea. There is a concomitant loss of cochlear dendrites and, to a lesser extent, of neuronal cell bodies that parallels closely the spatial distribution and magnitude of atrophy of the supporting cells of the organ of Corti. Wright et al[14] found a generalized and gradual reduction in the outer hair cell density with age, but with an additional loss in the elderly at the base and to a lesser extent at the apex. The apical loss is apparently restricted to persons over 70 years of age. In our cases, the apical hair cell loss is minimal and insignificant. For the inner hair cell loss, the decline is less marked but follows the same pattern.

Microscopists observed long ago that lipofuscin accumulates in the tissues of aged individuals; hence its popular synonym "wear and tear pigment" and its German name *Abnutzungspigment*.[15-17] Ishii et al[18,19] have shown that lipofuscin granules accumulate in the apical cytoplasm of the hair cells and supporting cells of the organ of Corti and the vestibular sense organs of the aging ear. It was not demonstrable in individuals under the age of 6 years, but increased in quantity as a function of age. The location of the lipofuscin granules correlated with the location of lysosomes and was therefore assumed to be a waste product of lysosomal activity. Lysosomes are rich in acid hydrolases, and at least 16 different enzymes have been identified in them.[20] Bennett[21] was the first to suggest that insoluble end products of metabolism accumulate in lysosomes. It would seem reasonable that these changes are a reflection of exhaustion of enzymatic activity and an important cause of cell death.

Case 1 meets our criteria for sensory presbycusis, that is, an uninterrupted loss of hair cells at the basal end involving at least a 10-mm area (Fig 1). Sensory presbycusis also appears as a component of mixed presbycusis in cases 14, 15, and 16 (Table 2).

Neural Presbycusis. Otte et al[10] counted the neurons in cochleae from 100 hearing ears from subjects whose ages spanned nine decades and in whom there was no evidence of disease affecting the neurons. The populations ranged from 36,918 for those in the first decade to 18,626 for those in the ninth decade, for a linear progressive loss of about 2,100 neurons per decade (Fig 2).

A loss in population of cochlear neurons in the presence of a functional end organ creates a distinctive pattern of auditory dysfunction characterized by a progressive loss of word discrimination in the presence of stable pure tone thresholds. Gaeth[22] has used the term phonemic regression to describe this phenomenon. Palva and Jokinen[23] found the word discrimination scores of patients over 60 years of age to be better in the left ear and attributed this to left cerebral dominance becoming manifest as the result of degenerative change in the auditory pathways. Elderly patients with rapidly progressive neural presbycusis often demonstrate associated diffuse degenerative changes of the central nervous system, exhibited by motor weakness and lack of coordination,

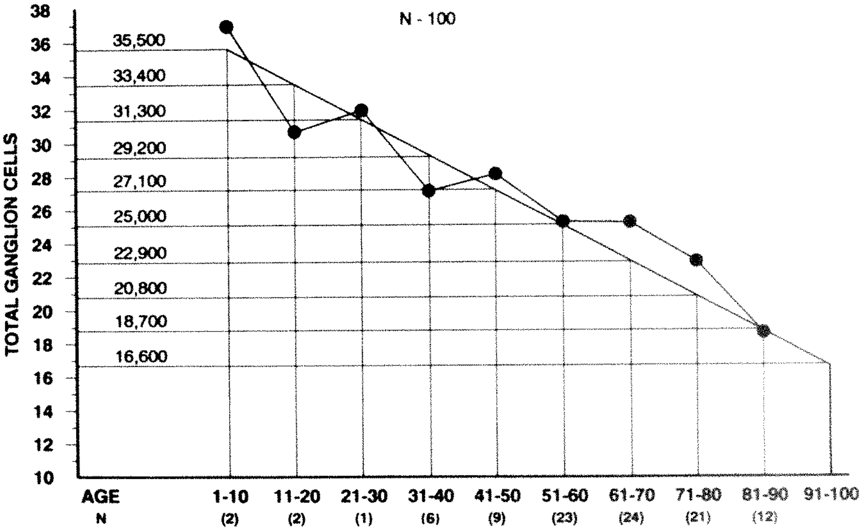


Fig 2. Cochlear neuronal populations as function of age. Means show uniform loss of about 2,100 neurons per decade. Mean for 10th decade is extrapolated.

tremors, irritability, loss of memory, and intellectual deterioration. Neural presbycusis is characterized by a progressive loss of neurons throughout the cochlea with only a very slight tendency to be more severe at the basal end.[24]

The loss of cochlear neurons is the most consistent pathologic change in the aging ear. It begins at an early age and continues throughout life. The pure tone thresholds are variable, and it seems quite clear they are not affected by neuronal loss until about 90% have disappeared.

The fact that the neurons that remain retain their dendritic fibers indicates that the missing neurons had undergone total degeneration (soma, axon, and dendrite).

Pauler et al[11] correlated neuronal populations with speech discrimination scores in 28 elderly persons. The neuronal counts were summed for four regions of the cochleae (0 to 6 mm, 6 to 15 mm, 15 to 22 mm, and 23 mm to the apex). A loss of cochlear neurons in the 15- to 22-mm region (the locus for the speech frequencies) correlated positively with a loss of word discrimination, whereas losses in the other regions did not.

Saccular degeneration involving both sensory and neural structures can occur concomitantly with cochlear degeneration as an aging process in both human subjects and animals.[25,26]

The neuronal losses in the auditory system that occur with aging are not limited to the periphery. Arnesen[27] calculated the total numbers of neurons in the ventral and dorsal cochlear nuclei from serial sections in six subjects 76 to 89 years of age. There was about a 50% loss of neurons compared to the numbers calculated for normal subjects by other investigators.

Cases 2, 3, and 4 meet our criteria for neural presbycusis, that is, a loss of 50% or more of cochlear neurons when compared to the mean for normal neonatal ears (Fig 3). Neural presbycusis also occurs as a component of mixed presbycusis in cases 12 to 16 (Table 2).

Strial Presbycusis. Atrophy of the stria vascularis is a common pathologic entity often affecting several members of a family. Typically, the hearing loss has an insidious onset in the third to sixth decades of life and progresses slowly. The clinical feature that distinguishes it from other types of presbycusis is the flat or slightly descending pure tone threshold audiometric pattern associated with excellent word discrimination scores. The findings indicate that ears with strial atrophy, when stimulated within their sensitivity ranges, are capable of accurate stimulus coding. Although the small increment sensitivity index test is usually positive for loudness recruitment, these patients do not complain of discomfort from loud sounds or of distortion. They respond well to the use of amplification and may be given a good prognosis for retaining useful hearing into old age.[2,5,28] Pauler et al[12] performed a quantitative study of strial tissue in 24 cochleae and found that the magnitude of strial atrophy correlated positively with the extent of elevation of pure tone thresholds.

Typically, there is patchy atrophy of the stria vascularis in the middle and apical turns of the cochlea.[8] There may be partial or complete loss of strial cells, sometimes with cystic structures and occasionally with basophilic deposits.

Takahashi[29] used the electron microscope to study the stria vascularis in temporal bones of persons over the age of 60. He demonstrated two types of atrophy: 1) a patchy type most severe in the apical and extreme basal regions and 2) a diffuse type often showing

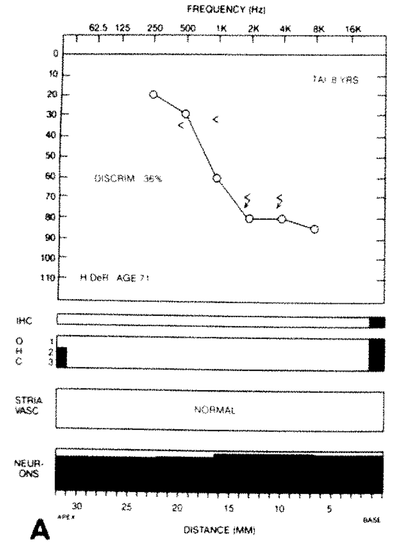
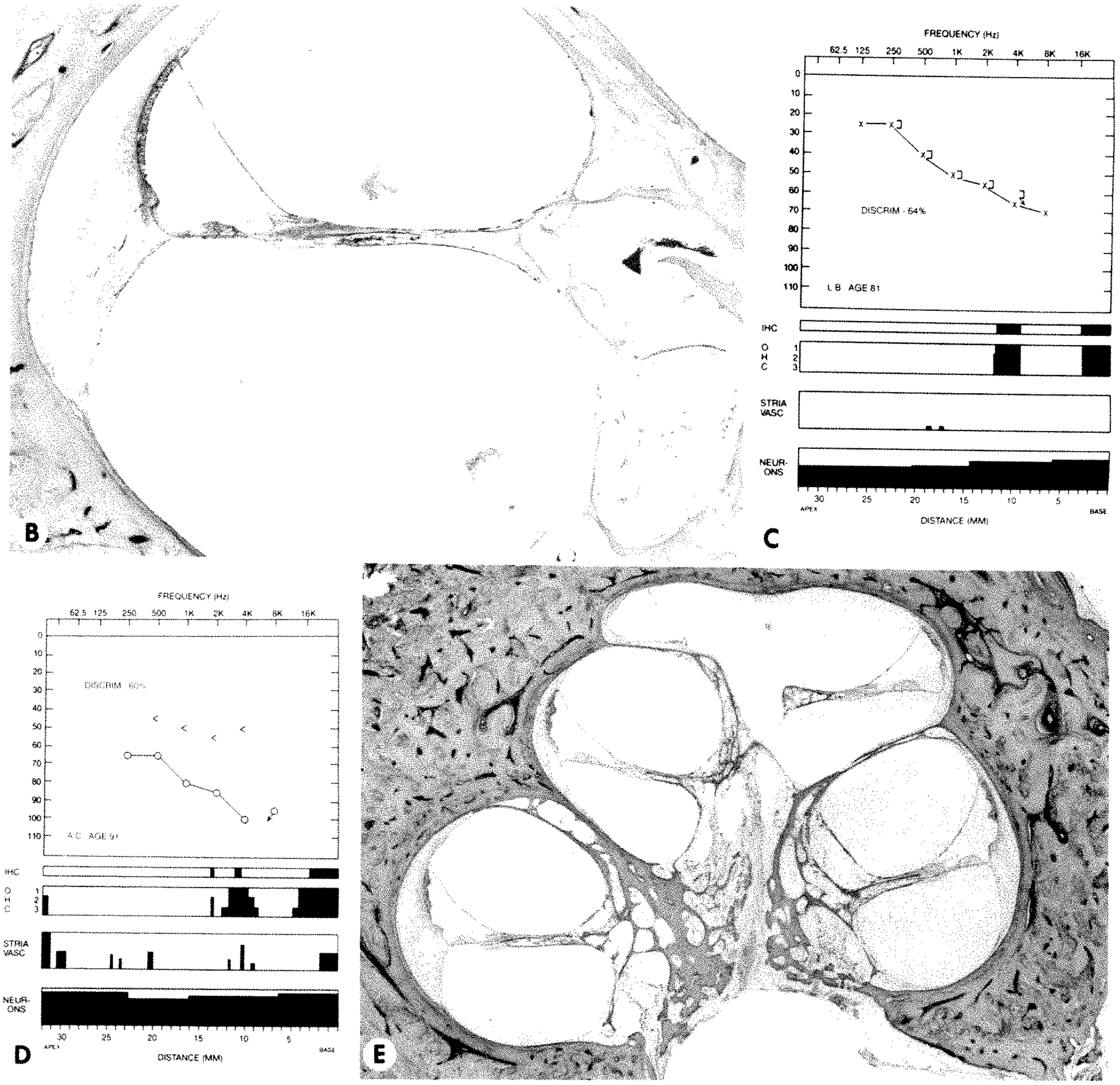


Fig 3. Neural presbycusis. A,B) (Case 2) 71-year-old man. A) Total neuronal loss is 89% of mean for neonatal normal. Sensory cell loss is minimal and stria is normal. TAI — test to autopsy interval. B) This view of 12-mm area shows normal organ of Corti and stria vascularis and near-total loss of cochlear neurons (x48). C) (Case 3) 81-year-old man. Total neuronal loss is 64% of mean for neonatal normal. Sensory and strial lesions are insignificant. There is 3-mm acoustic trauma lesion in 9- to 12-mm region. D,E) (Case 4) 91-year-old woman. D) Total neuronal loss is 83% of mean for neonatal normal. Four-millimeter sensory presbycusis and 3-mm acoustic trauma lesions are considered to be functionally insignificant. Apparent conductive hearing loss is believed to be artifact caused by pressure occlusion of ear canal by ear phone. E) This midmodiolar view shows normal organ of Corti and severe loss of cochlear neurons (x19).



normal strial thickness with large intercellular spaces that might not be visible by light microscopy. Kimura and Schuknecht,[30,31] using electron microscopy, found similar atrophic changes in surgical specimens of the cochleae of persons who had Meniere's disease and underwent labyrinthectomy and assumed they represented alterations of aging rather than Meniere's disease. All three cell layers of the stria were involved in various degrees and combinations; however, the marginal cells appeared to be most severely affected. In some regions of atrophy the stria consisted of only a layer of basal cells lining the endolymphatic space.

Probably the loss of strial tissue affects some quality of endolymph that results in a detrimental influence on the physical and chemical processes by which energy is made available to the sense organ. Experimental observations permit several interesting speculations on the functional significance of the stria vascularis. First, the stria vascularis appears to be the source of the positive 80-mV DC potential of the scala media.[32-34] Second, the stria vascularis has long been thought to be the site of endolymph formation.[35] Von Fieandt and Saxén,[36] using light microscopy, and later Engström et al[37] and Smith,[38] using electron microscopy, showed that the stria vascularis has morphologic features consistent with secreting organs in other parts of the body. Smith[38] has shown that the marginal cells that face the endolymph surround the capillary networks, with their basal surfaces exhibiting numerous extensions and infoldings in the basal plasma membrane that interdigitate with other cells, including the capillary endothelial cells. The implication of a secreting function is found in the fact that this type of structure is present in kidney tubules,[39] serous alveoli, choroid plexus, ciliary bodies, and secretory ducts of the submaxillary gland. Third, the tissues of the stria vascularis contain large amounts of oxidative enzymes that are required for glucose metabolism and that may be essential for the production of energy to support cochlear function.[40] These observations assign an important functional role to the stria vascularis.

Cases 5 to 8 meet our criteria for strial presbycusis, that is, a 30% or greater loss of strial tissue (Fig 4). Strial presbycusis also appears as a component in mixed presbycusis in cases 12, 13, 15, and 16 (Table 2).

Cochlear Conductive (Hypothetical) Presbycusis. Cochlear conductive presbycusis is differentiated from the indeterminate group by having linear descending audiograms. The diagnosis is derived by histologic exclusion of any consistent light microscopic explanation for the gradual descending pure tone threshold type of hearing loss. Cochlear conductive hearing loss usually first becomes evident in middle age, and the hearing loss shows almost equal increments of loss for each octave, with word discrimination scores inversely related to the steepness of the threshold gradient. Ramadan and Schuknecht[41] compared the temporal bone findings in two groups of elderly individuals with symmetric high-tone hearing losses. In the group that had gradually sloping threshold losses they were unable to detect a pathologic correlate by light microscopy, whereas the group with abrupt threshold losses had hair cell losses that were tonotopically consistent with those known to result from acoustic trauma.

Cases showing gradual descending threshold patterns, often characterized by straight descending audiograms, consistently fail to show any pathologic correlate. This type of hearing loss not only occurs as a manifestation of aging but is common in association with otosclerosis and Paget's disease of the temporal bone. Because the increments of loss show a linear relationship to frequency, it is speculated that the loss is caused by alterations in the resonance characteristics of the cochlear duct that determine frequency distribution. An attractive candidate for such an alteration is the basilar membrane, which features a nearly linear increase in width[42] and decrease in elasticity[43] from base to apex. It seems reasonable to attribute the linearity of the descending audiogram to degradation in compliance of the cochlear duct rather than to a spatially linear cellular loss or dysfunction. For these reasons, the term cochlear conductive presbycusis seems appropriate for these cases.

The question arises as to whether these hearing losses could be attributed to lesions of the cortical or subcortical auditory systems. It has been demonstrated in animal experiments that bilateral ablation of the auditory cortex does not alter pure tone thresholds. Large lesions at or below the level of the tectum produce pure tone threshold losses; however, transection of the brachium of the inferior colliculus and transection of the auditory commissures of the medulla have little or no effect on pure tone thresholds. In humans, evidence linking central lesions to a specific type of audiometric pattern is lacking. A concept has evolved among researchers that cortical and subcortical lesions of the auditory pathways affect the ability to discriminate complex acoustic patterns (eg, sound localization, discriminations involving pitch, loudness levels) but have little or no effect on pure tone thresholds.[44]

Mayer[45] suggested that impaired hearing of old age might be due to stiffening of the basilar membrane and supported his thesis with histologic sections that he interpreted as showing calcification of

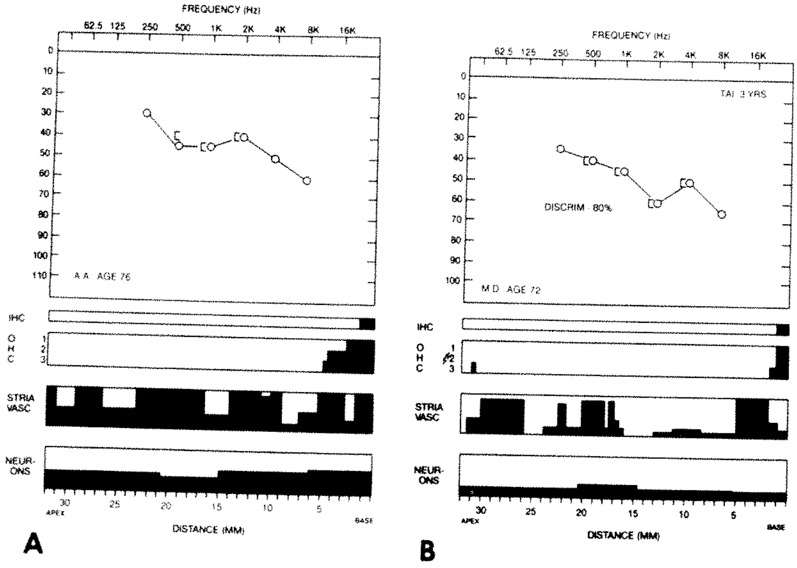
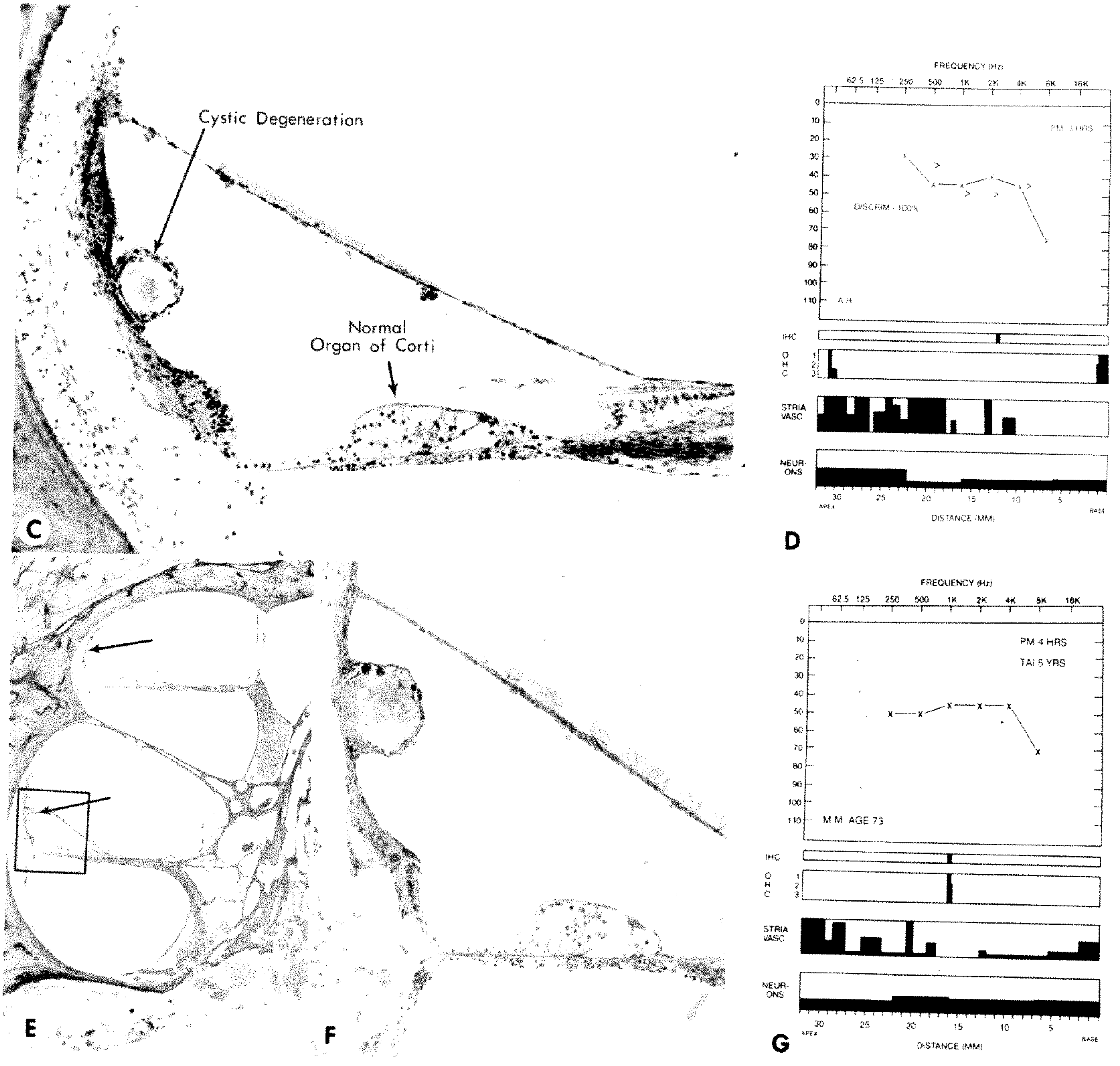
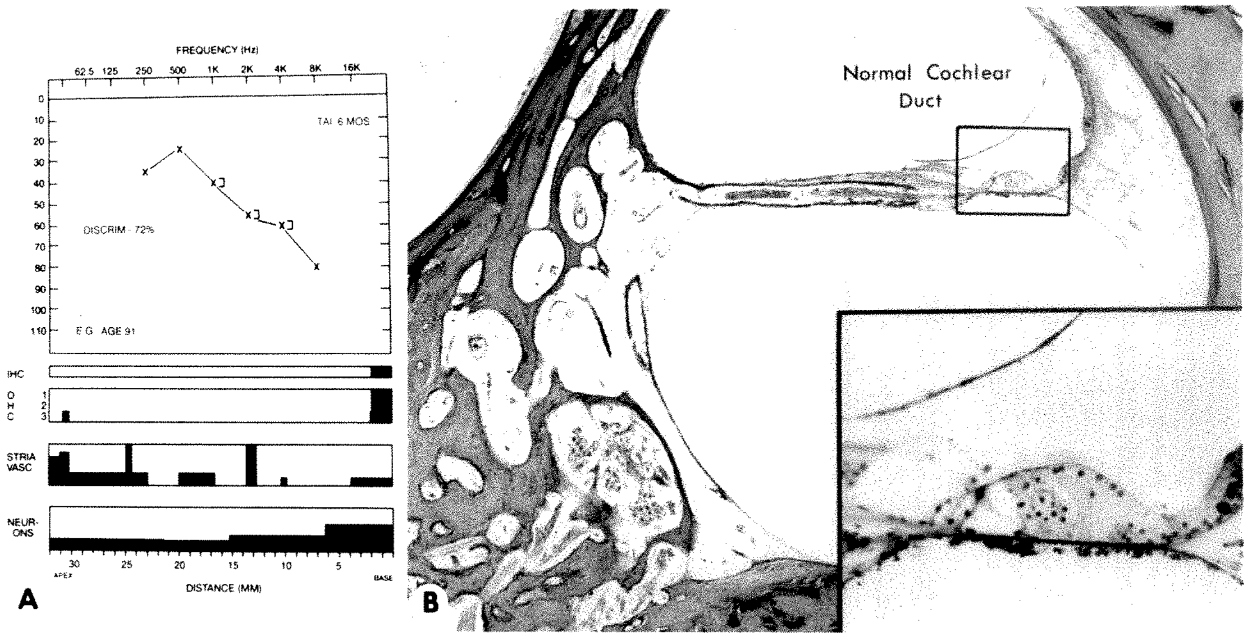


Fig 4. Strial presbycusis. A) (Case 5) 76-year-old woman. Loss of stria is estimated to be 77.1%. Losses of sensory cells (4 mm) and neurons (49%) are not significant by our criteria. B,C) (Case 6) 72-year-old woman. B) Loss of stria is estimated to be 51.2%. Losses of sensory cells (1.2 mm) and neurons (25%) are not significant by our criteria. C) This view of middle turn shows partial cystic degeneration of stria vascularis. Organ of Corti is normal (x119). D-F) (Case 7) 72-year-old man. D) Loss of stria is estimated to be 44%. Losses of sensory cells (1.2 mm) and neurons (30%) are not significant by our criteria. PM — postmortem. E,F) This view shows severe loss of strial tissue (arrows in E) in otherwise normal cochlear duct. E) Magnification x14. F) Boxed area of E (x112). G) (Case 8) 73-year-old woman. Strial loss is judged to be 35.4%. Sensory and neuronal losses are insignificant by our criteria.





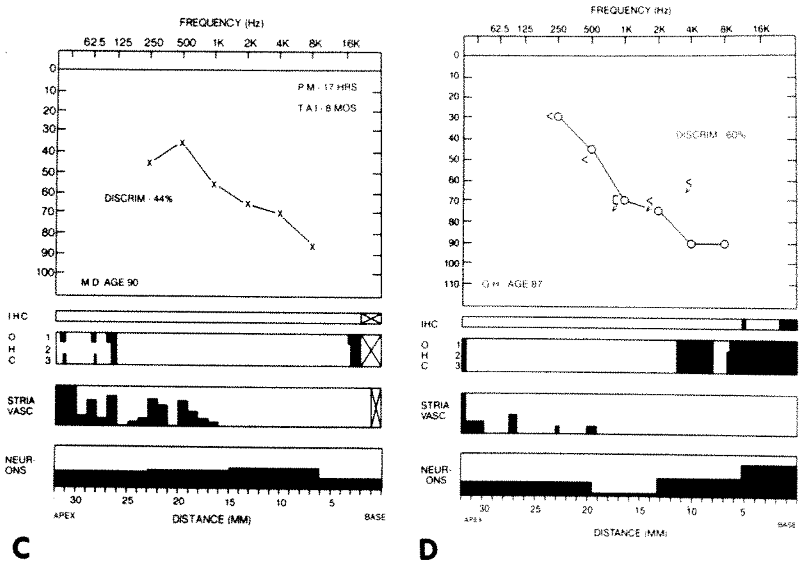


Fig 5. Cochlear conductive presbycusis. A,B) (Case 9) 91-year-old man. A) Gradual descending pure tone threshold. Sensory cell lesion is 2 mm, strial loss is 24.9%, and neuronal loss is 34%, none of which meet our criteria for significant pathologic change. B) Normal cochlear duct in 11-mm area, which serves 4-kHz frequency and for which there is 60-dB hearing loss (x39). Inset) Magnification x147. C) (Case 10) 90-year-old woman. Gradual descending pure tone threshold. Sensory cell lesion at basal end is 3 mm, strial loss is 23.2%, and neuronal loss is 42%, none of which qualify as significant pathologic lesion by our criteria. D) (Case 11) 87-year-old man. Gradual descending pure tone threshold. There is 6.5-mm sensory presbycusis lesion, loss of 6.3% of stria and 41% of neurons, none of which meet our criteria for significant pathologic change. Loss of outer hair cells in 8- to 11.5-mm region can be attributed to acoustic trauma.

the basilar membrane. Crowe et al found partial atrophy of the cochlear nerve to the basal turn to be a prominent lesion in the majority of ears with descending audiometric thresholds, but noted that "some ears with the gradual type of loss do not have sufficient degree of nerve atrophy to explain the impairment of hearing."[46(p374)] They also reported finding hyalinization and deposition of calcium salts in the basilar membrane at the basal end of the cochlea to be more common in patients with descending audiometric patterns than in normal ears or ears with abrupt high-tone hearing losses. Covell and Rogers[47] and Pestalozza et al,[48] in studies on senile guinea pigs, found that the losses in cochlear microphonic response were greater than could be attributed to hair cell changes and suggested that conductive lesions might be present. Glorig and Davis[49] accept the idea of an inner ear conductive impairment as being logical. In support of this concept they observed that loudness recruitment is absent in many cases of presbycusis.

Nomura[50] found neutral fat and cholesterol in the filamentous structure of the pars pectinata of the basilar membrane in 9 of 20 aged patients. He considered this lipidosis of the basilar membrane to represent an alteration of aging that might cause hearing loss. Kraus[51] has shown that the basilar membrane of guinea pigs exhibits a decrease in density as a function of aging; this finding supports the concept of a physical-chemical alteration in its structural characteristics. Support for the concept of an inner ear conductive hearing loss is found in the light and electron microscopic study of a single cochlea by Nadol.[52] His report concerned an 81-year-old man

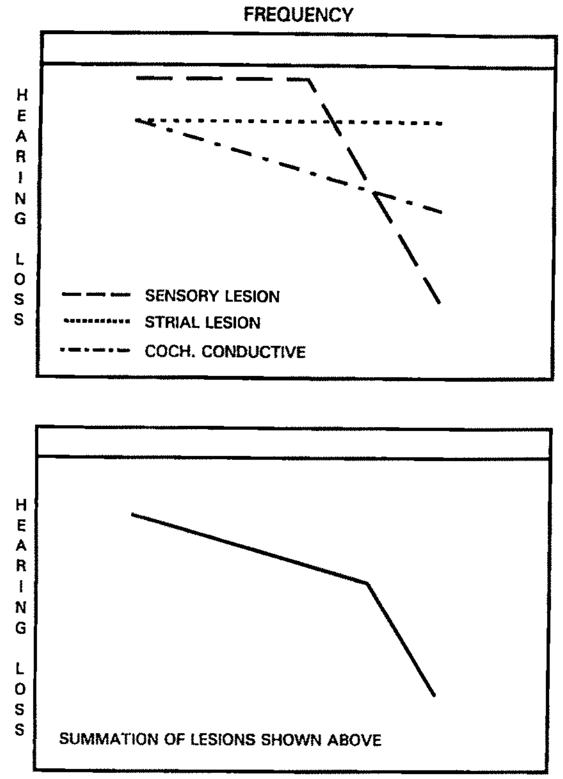


Fig 6. How additive effects of different pathologic types of presbycusis occurring in same cochlea can produce variety of pure tone threshold audiometric profiles.

who by history had a progressive bilateral hearing loss. In addition to severe atrophic changes in the organ of Corti, there was marked thickening of the basilar membrane due to an increase in the number of fibrillar layers.

Cases 9, 10, and 11 meet the criterion of having gradual descending pure tone thresholds as defined in Materials and Methods, and do not meet our criteria for significant pathologic changes (Table 2 and Fig 5). Cochlear conductive presbycusis may also have occurred as a component of pathologic changes in cases 3, 13, and 16.

Mixed Presbycusis. Whereas the four classic types of presbycusis occur in isolation often enough to be recognized as clear clinical and/or pathologic entities, many presbycusic hearing losses present as mixtures of these types. If we accept the existence of a cochlear conductive presbycusis and assume that the different types of presbycusis are additive, the audiometric pattern should reflect the underlying pathologic changes in the cochlea (Fig 6). For example, the combination of sensory and strial presbycusis would cause an abrupt high-tone hearing loss superimposed on a flat threshold loss. A sensory and cochlear conductive presbycusis would present as an abrupt high-tone hearing loss superimposed on a descending threshold audiogram. Strial and cochlear conductive presbycusis would manifest a gradual descending slope superimposed on a flat threshold loss.

Cases 12 to 16 meet the criterion of having significant pathologic changes in more than one cochlear structure (Table 2 and Fig 7).

Indeterminate Presbycusis. Histologic study reveals cases (possibly 25% of all presbycusis cases), mostly with a flat and/or abrupt high-tone hearing loss, that show no consistent pathologic correlate. Audiometrically, they fit into the strial and/or sensory presbycusis categories. It may be speculated that these indeterminate cases represent similar patterns of cochlear dysfunction but are caused by impaired cellular function rather than cellular attrition and therefore are not detectable by light microscopy.

Changes that would elude detection by light microscopy are 1) alterations in intracellular organelles that would impair cell metabolism, 2) diminished numbers of synapses on the hair cells, and 3) chemical alterations in the endolymph. In human postmortem temporal bones, these alterations, if present, could not be identified by light microscopy. Another possibility is alterations in the auditory pathways in the brain. Cases 17 to 21 meet the criterion of having indeterminate presbycusis, that is, no significant pathologic change, as previously defined, to explain the hearing losses (Table 2 and Fig 8).

Other Cochlear Changes With Aging. Almost nothing is known of the effect on hearing of aging alterations in the spiral ligament, Hensen's cells, Reissner's membrane, the tectorial membrane, or the limbus.

Alterations in the spiral ligament are quite constant as an aging process, beginning in childhood and progressing throughout the life of the individual.[53] When these changes occur in a moderate degree they are compatible with normal hearing. The changes appear to be as consistent a function of aging as those that occur, for example, in the skin. The atrophy is most severe in the apical half of the cochlea and is progressively less severe toward the basal end. The earliest alteration is a loss of fibrocytes in the region adjacent to the attachment of the basilar membrane (basilar crest). As the atrophy progresses, a zone of acellularity develops in the midportion of the spiral ligament. There appears to be a loss of fibrocytes, as well as a migration of fibrocytes toward the margins of the ligament. With further change, two distinct zones are seen: a larger, internal zone, remarkable for its acellularity and cystic spaces, and a smaller, external zone containing scattered fibrocytes in a fibrillar stroma. Commonly there is a dense layer of closely packed fibrocytes at the interface between

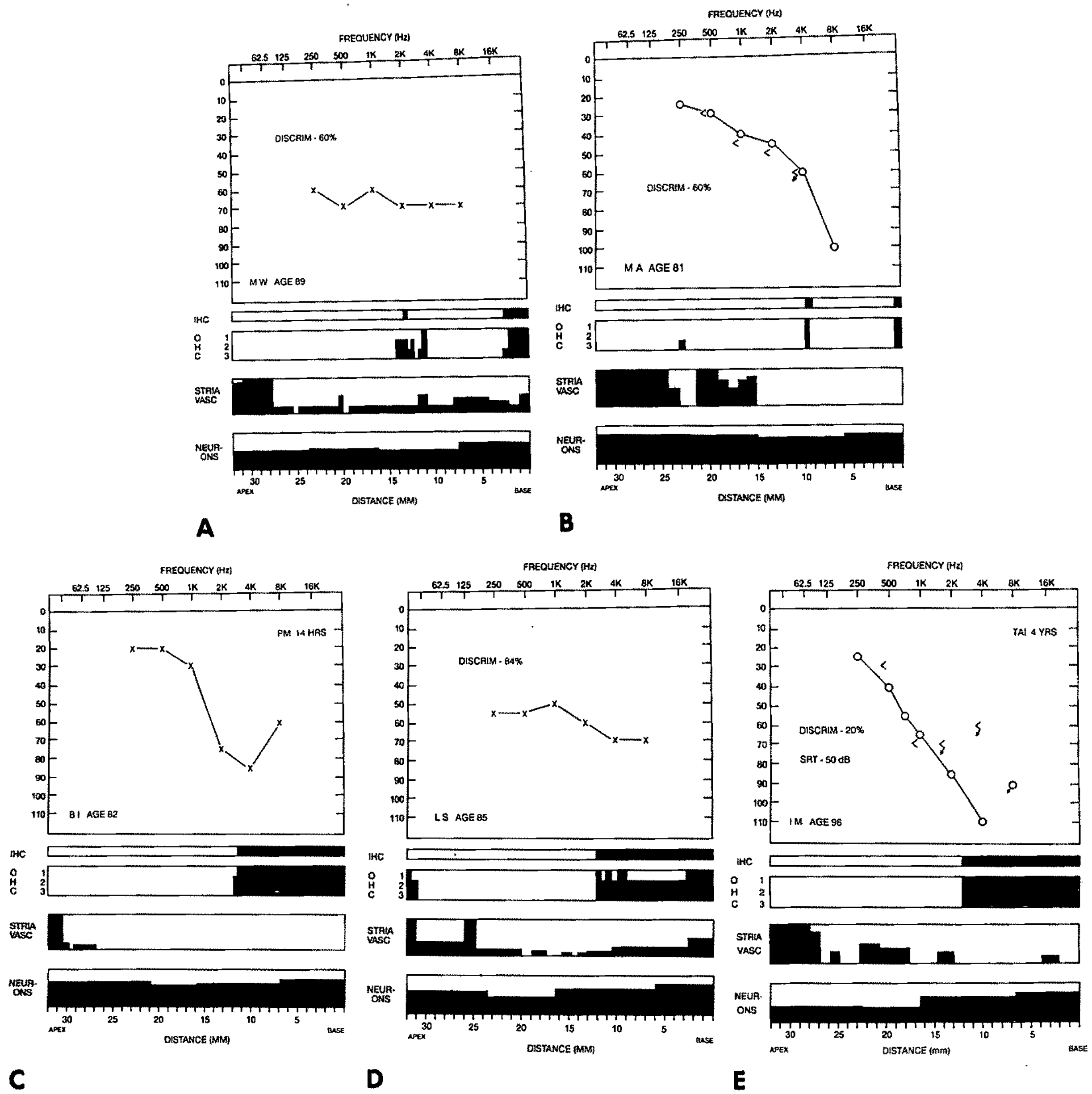


Fig 7. Mixed presbycusis. A) (Case 12) Strial and neural, in 89-year-old woman. There is flat pure tone threshold loss of 60 to 70 dB. There is 40.3% loss of stria and 56% loss of neurons, both of which meet our criteria for pathologic significance. Two-millimeter sensory presbycusis and 3.5-mm acoustic trauma lesions are insignificant. B) (Case 13) Strial and neural, in 81-year-old woman. There is gradual descending threshold pattern that does not satisfy our criteria for cochlear conductive presbycusis in terms of magnitude (see text). Nonetheless, mild cochlear conductive lesion might be present. Strial loss is 42.9% and neuronal loss is 73%, both of which are significant by our criteria. C) (Case 14) Sensory and neural, in 82-year-old man. There is abrupt high-tone hearing loss for frequencies above 1 kHz. Audiogram made 11 years prior to death shows upturn at 8 kHz that suggests that this man, construction riveter, had distinct acoustic trauma lesion at that time, which later merged with sensory presbycusic lesion to produce uninterrupted 12-mm lesion. There is 65% loss of neurons. Both sensory and neural lesions meet our criteria of pathologic significance, whereas strial loss of 7.9% does not. D) (Case 15) Sensory, strial, and neural, in 85-year-old woman. There is flat 50- to 70-dB pure tone threshold. Cochlea shows 12-mm sensory lesion, 33.1% loss of stria, and 56% loss of neurons, all of which are pathologically significant as defined by our criteria. E) (Case 16) Sensory, strial, neural, and cochlear conductive, in 96-year-old woman. This cochlea meets our criteria for all four types of presbycusis. Descending pure tone threshold meets our requirements for cochlear conductive presbycusis, that is, gradient of at least 50 dB over 5-octave range with no more than 25-dB difference between any two adjacent frequencies. There is also 12-mm sensory lesion, loss of 31.2% of stria, and loss of 55% of neurons, all of which are pathologically significant by our criteria.

these zones. All of these changes can be found in normal-hearing ears, but we have the impression that they are more frequent in ears exhibiting descending audiometric patterns. As the spiral ligament shrinks, the configuration of the cochlear duct is altered. Commonly the basilar membrane retains its continu-

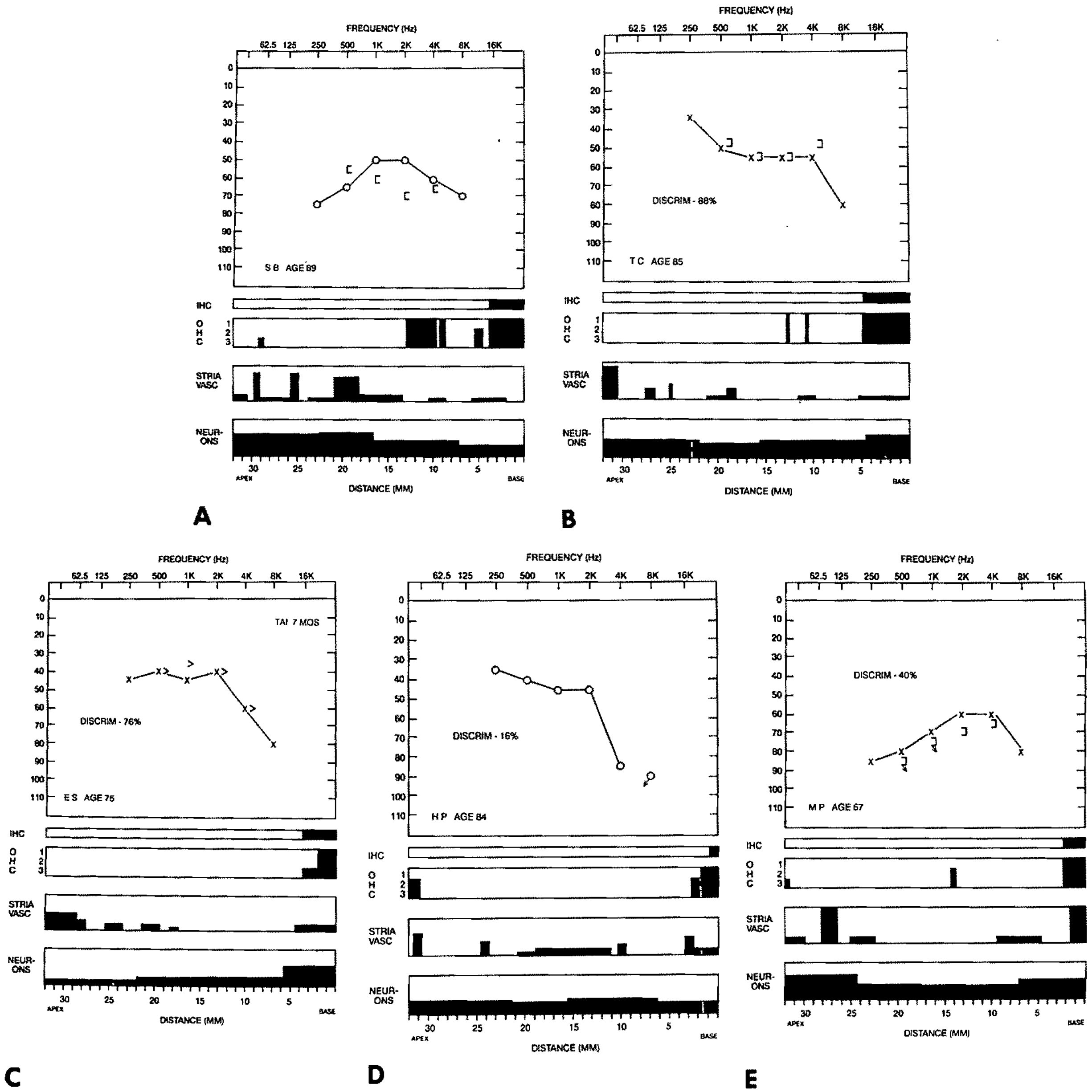


Fig 8. Indeterminate presbycusis. A) (Case 17) 89-year-old man. There is flat pure tone hearing loss. There is 4-mm sensory presbycusic lesion, 20.7% loss of stria, and 49% loss of cochlear neurons, none of which reach our criteria for pathologic significance. There is loss of outer hair cells in 9- to 14-mm region that is consistent with acoustic trauma. B) (Case 18) 85-year-old woman. Mildly descending pure tone threshold is present. There is 5-mm sensory lesion, 12.4% loss of stria, and 46% loss of neurons, none of which reach pathologic significance by our criteria. C) (Case 19) 75-year-old woman. Pure tone thresholds show flat loss up to 2 kHz and descending pattern above 2 kHz. There is 4-mm sensory lesion, 15.6% loss of stria, and 28% loss of neurons, none of which reach pathologic significance by our criteria. D) (Case 20) 84-year-old man. There is mild decreasing pure tone threshold for frequencies up to 2 kHz and abrupt severe loss for higher frequencies. There is 2.5-mm sensory lesion, 18% loss of stria, and 35% loss of neurons, none of which reach pathologic significance by our criteria. E) (Case 21) 67-year-old woman. There is severe hearing loss with slightly ascending pure tone threshold. Cochlea shows 2.1-mm sensory lesion, 17.9% strial loss, and 49% loss of cochlear neurons, none of which reach pathologic significance by our criteria.

ity with the structures of the lateral wall by a thin layer of ligamentous tissue that converts the cochlear duct from a triangular to a flattened shape. Sometimes the basilar membrane separates from the lateral wall by a break in the area between the basilar crest and the spiral prominence. This brings the endolymphatic space into continuity with cystic spaces in the spiral ligament. When this happens, the only remaining connection of the basilar membrane with the bony cochlear wall is a thin layer of spiral ligament tissue bordering the scala tympani. The functional effects of lesions of this type are not known. Rupture of the cochlear duct and profound hearing loss may be the ultimate, but rare, end result of advanced atrophy of

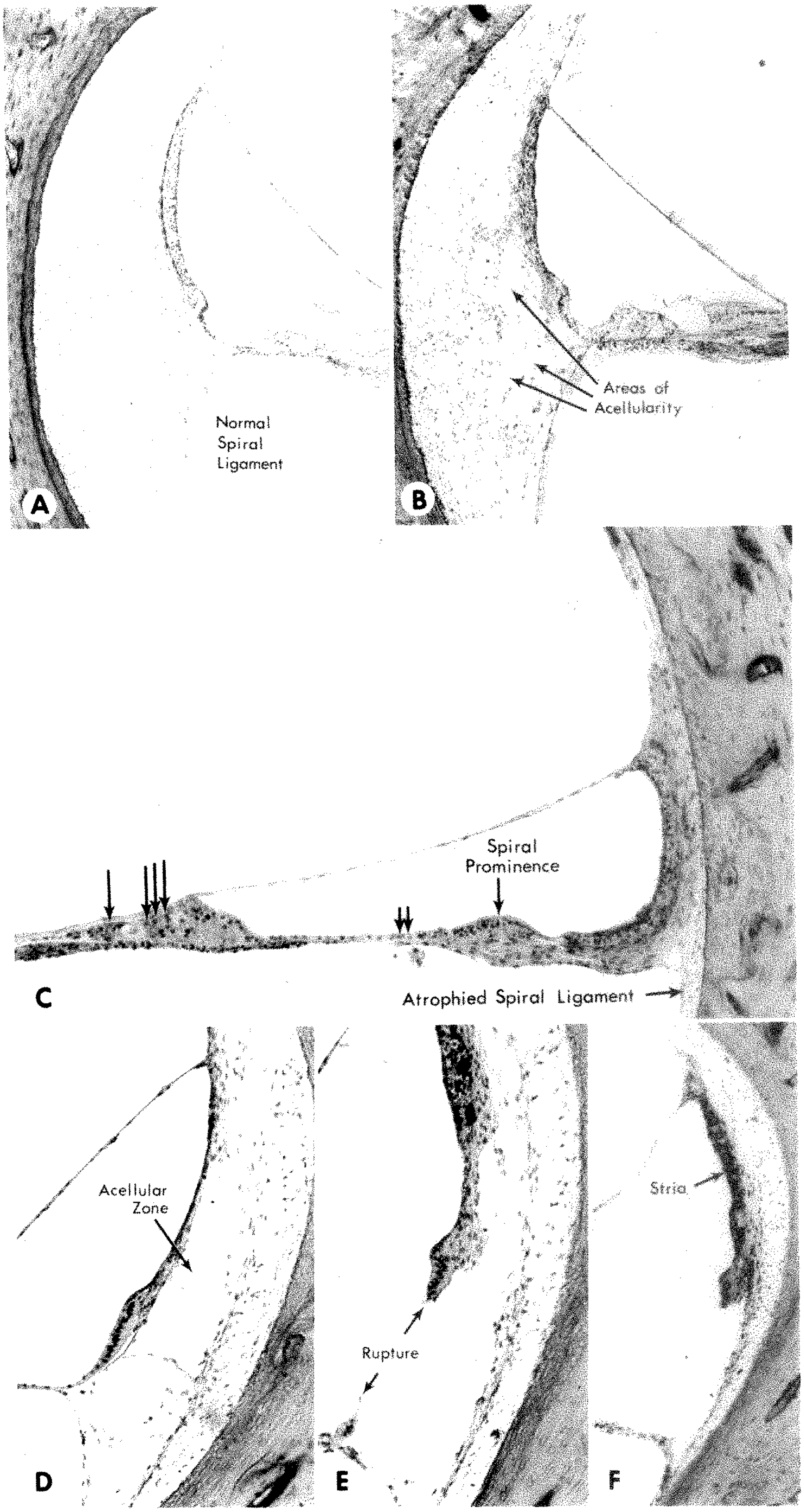


Fig 9. Aging changes in spiral ligament. A) Normal spiral ligament in newborn boy (x71). Fibrocytes are evenly distributed throughout structure. B) Atrophy of spiral ligament in 77-year-old woman with no history of hearing loss (x71). Spiral ligaments of basal turns show areas of decreased cellularity, while adjacent areas show strands of increased cellularity. This is normal aging process and appears to be result of migration and clustering of fibrocytes. Often, densest accumulation of fibrocytes is in substrial area. Structures of cochlear duct appear normal. C) Atrophy of spiral ligament in 36-year-old man who had not complained of deafness (x136). Both cochleae show severe atrophy of spiral ligaments in middle and apical turns. Cochlear duct in 18-mm region of left ear is shown here. Organ of Corti shows postmortem autolysis; however, hair cells are present (long arrows). As spiral ligament shrinks, radial dimension of cochlear duct becomes elongated and shallow. Spiral prominence and part of stria vascularis assume same plane as basilar membrane. Double arrow marks position of basilar crest, which is normal area of attachment of basilar membrane to spiral ligament. D-F) Atrophy of spiral ligament in 63-year-old woman who had not complained of deafness. Both cochleae show severe atrophy of spiral ligaments in middle and apical turns. Three views are shown from upper middle turn of left cochlea. D) Spiral ligament shows inner acellular zone and outer cellular zone (x107). At interface of these zones there is increase in density of fibrocytes. Stria vascularis is atrophied; however, this pathologic change bears no relationship to spiral ligament atrophy. E) In area adjacent to D, rupture has occurred in lateral wall of cochlear duct between basilar membrane and spiral prominence, creating confluence of endolymphatic space with acellular zone of spiral ligament (x119). F) Slightly more apicalward, stria vascularis has retracted outward and is attached to outer cellular zone of spiral ligament (x73). Organ of Corti in this ear shows postmortem autolysis but retains hair cells. It is not known what effect, if any, this pathologic change in spiral ligament might have on hearing. Certainly, these atrophic changes are pathologic, but it cannot be determined with certainty whether rupture is pathologic event or preparation artifact caused by shrinking effect of fixative solutions on already fragile spiral ligament.

the spiral ligament. In assessing morphologic changes of this magnitude, it is important to consider the possible role of postmortem autolysis and preparation artifact as contributors to these changes (Fig 9).

Atrophy of Reissner's membrane as a condition of aging has been described by Johnsson[54] and by Watanuki et al.[55] It is characterized histologically by vacuoles and blebs in the epithelial cell layer, thinning of the membrane as a consequence of loss of cells, and irregularity in the distribution of both the epithelial and mesothelial cell layers. Rupture of Reissner's membrane may result from these atrophic changes. The membrane may collapse and distort the cytoarchitecture of the organ of Corti and entrap the tectorial membrane. These changes can occur without imposing a loss of hair cells or neurons, but presumably would interfere with the motion mechanics of the organ of Corti.

DISCUSSION

The findings of this study show that the basic tenet of four pathologic types of presbycusis is valid. It is apparent, however, that many aging ears show combinations of pathologic types. Whereas a particular type may predominate, its occurrence in total isolation from the others is the exception rather than the rule.

A loss of sensory cells appears to be the least important cause for hearing impairment caused by aging. The area of hair cell loss at the basal end of the cochlea may be a common cause for a threshold elevation for 8 kHz, but rarely does the degenerative change extend apicalward far enough to involve the speech frequencies. When a high-tone loss involves 4 kHz, it usually signals a lesion complicated by acoustic trauma.

The most consistent pathologic change in the aging cochlea is a loss of cochlear neurons. That the neurons that remain retain their dendritic fibers indicates that the missing neuronal units had undergone total degeneration, including dendritic fiber, soma, and axon — a condition termed primary neuronal degeneration. When a partial loss of neurons occurs as a secondary effect of atrophy of the organ of Corti, many (and often most) of the remaining neurons have lost their dendritic fibers — a condition termed retrograde or secondary neuronal degeneration. The only occasion in which retrograde neuronal degeneration occurs as a function of aging is severe sensory presbycusis, in which atrophy of the supporting cells of the organ of Corti causes injury to the dendritic nerve fibers. In this case the neuronal loss does not aggravate the hearing loss, because it is masked by the dominating sensory lesion.

Primary neuronal degeneration has to be in the range of a 90% loss to have an effect on pure tone thresholds — a condition also noted in animal studies.[56] Neuronal losses appear to have their greatest effect in degrading the capability for word discrimination; however, losses of up to 50% are compatible with normal or only slightly reduced word discrimination scores.

Atrophy of the stria vascularis, when it occurs as an effect of aging, is located principally in the apical half of the cochlea. It has a patchy distribution, and all cell layers are degenerated in the involved areas. The audiometric patterns show a strong tendency toward flat pure tone thresholds. We suspect that some biochemical or energy-providing function of endolymph is affected by strial degeneration, which causes a functional deficit throughout the endolymph of the cochlear duct, resulting in flat audiometric patterns.

In spite of every attempt to establish meaningful relationships between the cochlear disorders and corresponding audiometric abnormalities, including invoking the concept of a cochlear conductive lesion, there are about 25% of hearing losses of aging, mostly with flat audiograms, that cannot be explained by light microscopic study.

CONCLUSIONS

1. Sensory cell losses are the least important cause of hearing loss in the aged. The losses are located in the basal end of the cochlea and cause abrupt high-tone hearing losses; however, the lesions are often merged with acoustic trauma lesions and cannot be clinically or histologically differentiated from them.

2. Neuronal losses are a constant and predictable expression of the aging cochlea; however, in some cases the losses exceed the norm for age, thereby further degrading the capability for word discrimination.

3. Atrophy of the stria vascularis occurring as a predominant lesion of the aging cochlea is located principally in the apical and middle turns and characteristically is associated with hearing losses showing flat audiometric patterns.

4. Light microscopic studies have failed to reveal a pathologic correlate to explain the gradual descending pure tone threshold, and a cochlear conductive disorder seems the most logical explanation.

5. Many aging ears show significant involvement of two or more of the four basic pathologic types of presbycusis. The types appear to be additive, resulting in a wide spectrum of alterations in pure tone thresholds and word discrimination scores.

6. There is an indeterminate group consisting of about 25% of cases that do not meet the criteria for any of the four types of presbycusis, and it is assumed that the cause lies in morphological, chemical, and/or physical alterations that cannot be identified by light microscopic study.

7. The effectiveness of amplification for presbycusis depends in great part on the functional status of

these several cochlear structures. Their pathologic condition will determine pure tone threshold elevation, threshold gradients of the auditory spectrum, and word discrimination ability. Successful hearing aid design for presbycusis depends on providing comfortable amplification and control of background noise, but it is limited by pathophysiologic cochlear deficits that degrade stimulus coding. In the current state of medical knowledge, the degenerative changes of the aging cochlea are unyielding to medical therapy.

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THREE-DIMENSIONAL ANALYSIS OF MURINE LARYNGEAL DEVELOPMENT

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THREE-DIMENSIONAL ANALYSIS OF MURINE LARYNGEAL DEVELOPMENT

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BRONX, NEW YORK

This study defines the critical stages in the prenatal development of the mouse larynx. Three-dimensional computer-generated solid model reconstructions display the changing anatomic relationships during laryngeal development from the earliest respiratory primordium to the mature laryngeal vestibule. These reconstructions demonstrate that the laryngeal vestibule forms as a result of a cephalocaudal separation of the epithelial lamina, thereby establishing communication between the laryngeal cecum and the pharyngoglottic duct. These results support the hypothesis that the cause of congenital supraglottic and glottic malformations is an incomplete separation of this epithelial lamina. The infraglottic lumen remains patent during laryngeal development. Continued lengthening of the foregut between the infraglottis and the carina forms the trachea. This is contrary to the 19th-century concept of an ascending tracheoesophageal septum. Formation of the laryngeal cartilages, muscles, and innervation is portrayed by the reconstructions; with minor variations, they correlate closely with earlier wax reconstructions of human laryngeal development. Future research with this animal model can provide additional insights into the complex mechanisms involved in congenital malformations of the larynx.

KEY WORDS — congenital laryngeal atresia, epithelial lamina, infraglottis, laryngeal cecum, pharyngoglottic duct, primitive laryngopharynx, three-dimensional reconstruction.

INTRODUCTION

The information available on laryngeal embryology is limited to descriptive anatomic studies. Observations and theories concerning normal and abnormal laryngeal development have been based primarily on histologic sections of human embryos and/or wax reconstructions.

Conflicting concepts of the origin of the laryngeal cavity and vestibule have been proposed. These concepts will be presented in greater detail in the Discussion. The main controversy appears to be the determination of the level of the cephalic end of the trachea. Kallius[1] (1897), Frazer[2] (1910), and Walander[3] (1950) accepted the theory of His[4] (1885), which described an ascending tracheoesophageal septum that began behind the respiratory diverticulum and reached the level of the fourth pharyngeal pouch (pharyngeal floor) in the process of forming the trachea. His assumed that the cephalic end of the trachea was located at the level of the pharyngeal floor. Conversely, Zaw-Tun[5] (1982) proposed that the cephalic end of the trachea was separated from the pharyngeal floor by a foregut segment called the primitive laryngopharynx, and he assumed the cephalic end of the supraglottis to be at the level of the pharyngeal floor. In view of the persistence of these opposing concepts, there has been no consensus of opinion regarding the mechanism of congenital laryngeal malformations.

The purpose of this paper is threefold: 1) to identify the basic embryologic events in the formation of the murine laryngeal vestibule, skeleton, and musculature, 2) to determine if the critical stages in the development of the mouse larynx parallel the developmental events that have been described for the human larynx, and 3) to attempt a correlation of the congenital malformations described for the human larynx (eg, posterior laryngeal clefts, supraglottic and glottic stenosis) with critical periods in the development of the mouse larynx.

The mouse was chosen for this study for the following reasons: 1) laryngeal development is essentially uniform in all mammals, 2) the genetic analysis of murine development is well defined, and 3) embryos of a predetermined age can be easily and accurately staged. Histologic sections and computer-generated three-dimensional (3-D) solid model reconstructions are presented to facilitate an understanding of the changing anatomic relationships.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The embryonic material used in this study was

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from CBA/J and C57 BL/6J strains of normal mice that were cross-mated to produce CBA/C57 hybrid embryos. Gestational age was estimated by the vaginal plug technique, with the day of plug being designated as gestation day 1. Pregnant female mice were killed painlessly by cervical dislocation on the desired gestational days. The actual ages of the embryos were determined by somite count for the first 13 days of gestation. Older stages were determined by somite count plus external features (Theiler,[6] 1972). Microsurgical technique was used to obtain en bloc specimens of the laryngopharyngeal region. Transverse sections at the oral commissure cephalically and the clavicular level caudally defined the limits of the dissection. Laryngopharyngeal specimens were obtained from embryos of 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 14, 15, 16, 17, and 18 days' gestation.

HISTOLOGY

Specimens were fixed in Perfix (Fisher) at 4°C for a variable length of time, depending on the gestational age of the specimen, and then processed for paraffin embedding. Serial sections were cut at 7 µm in axial, coronal, and sagittal planes for each gestational age, and stained with a standard methylene blue procedure. Analysis of histologic specimens was performed at a light microscope level.

STERECON RECONSTRUCTIONS

STERECON is a computer graphics system designed to allow tracing of structural contours in three dimensions, and the creation of 3-D reconstructions from the contours.[7,8] It is applicable to images obtained through a wide range of light and electron microscopic techniques. The system was designed at the National Institutes of Health Biotechnology Resource, located at the Wadsworth Center for Laboratories and Research in Albany, NY. Two-dimensional images obtained from consecutive sections from the above histologic series were used to generate 3-D models.

Photographic transparencies of each section were projected onto a screen that was the same size as a high-resolution computer monitor. A half-silvered mirror was used to superimpose the projected images onto the monitor. By means of a digitizing tablet, color-coded lines were then drawn on the monitor outlining the structures of interest: epithelial lining of the foregut, foregut lumen, muscles, cartilages, arteries, etc. The resulting contours were stored in a data base and then used to form wire-frame models of the structures by using the MOSAIC portion of MOVIE.BYU.

This work was performed on a Sun work station with a Rastar Technologies Graphics terminal. The wire-frame models were then transferred to a Silicon Graphics work station and rendered as solid, shaded reconstructions with Wavefront Technologies Visualizer software. This allowed anatomic structures to be viewed in continuity, in any desired orientation, and in relation to any other given structure. In addition, internal structures could be visualized by sectioning the model in various planes, making the external structures transparent. Figure 1 is a color key that designates the different colors used for each anatomic structure in the 3-D reconstruction.

OBSERVATIONS

This section describes the morphologic changes in the laryngopharyngeal region that occur at each stage of development. Each sequential stage of development has the appearance of specific morphological features that did not exist at the previous developmental level. The important characteristic features of each stage are identified and an attempt is made to follow the continued presence of new structures, or their disappearance throughout later stages. The staging system used by Zaw-Tun[5] and Zaw-Tun and Burdi[9] was adopted as the convention for classifying observations made in this study.

Developmental arrests can lead to specific congenital anomalies of the larynx. For example, an interruption in the development of the laryngeal vestibule will lead to supraglottic and glottic stenosis (webs). Interruption in the development of the mesodermal anlagen of the larynx can give rise to the variable degrees of laryngotracheoesophageal clefting. The changes that occur to the laryngeal vestibule are described from stage 11 to adulthood. In addition, starting at stage 14, the changes occurring in the mesodermal anlagen of the larynx are described.

Three anatomic landmarks are critical to an understanding of the development of the laryngeal vestibule: the fourth pharyngeal pouch, the infraglottis, and the primitive laryngopharynx.

Fourth Pharyngeal Pouch. The median slit in the pharyngeal floor that gives rise to the larynx is at the same level as the fourth pharyngeal pouch. Controversy exists in the literature as to whether this slit represents the opening into the trachea,[1] the glottis,[2] or the supraglottis.[5,9] In this developmental series, the fourth pharyngeal pouch appears as early as stage 11, and is last seen in stage 16. After the disappearance of the fourth pharyngeal pouch, the level of the median slit in the pharyngeal floor can be identified between the enlarging arytenoid swellings, which originate from the pharyngeal floor and appear by stage 14.

- ⬜ epithelium
- 🟪 lumen
- 🟥 artery
- 🟪 condensed tissue
anlage of developing laryngeal muscle and cartilage
- 🟩 epiglottis
- 🟨 arytenoid swelling
nerve
- 🟫 hepatic anlage
muscle
- 🟦 cartilage

Fig 1. Color key for tissue types in three-dimensional (3-D) reconstructions.

Infraglottis. The shape of the infraglottic lumen has a characteristic appearance when sectioned in both a sagittal plane (it appears as an inverted triangle) and in a coronal plane (it appears as an upright triangle). This conical shape is consistent with the adult cricovocal membrane, or conus elasticus. During normal murine embryogenesis, the lumen of the infraglottis always remains patent. The infraglottis is derived from the cephalic aspect of the respiratory diverticulum. The infraglottis is just caudal to the level of the rima glottidis. This landmark is useful in understanding changes of the adjacent laryngeal vestibule. The infraglottis is initially present in stage 14.

Primitive Laryngopharynx. During stages 11 through 15, the primitive laryngopharynx is the region of the foregut that extends from the level of the fourth pharyngeal pouch to the entrance of the respiratory diverticulum, or the primitive pharyngeal floor. After stage 15, the fourth pharyngeal pouch, which becomes a fistulous tract, loses its continuity with the foregut. The arytenoid swellings, however, serve as a useful landmark in lieu of the fourth pharyngeal pouch, since they, too, originate from the pharyngeal floor. In addition, the respiratory diverticulum has already differentiated into the infraglottis region by stage 14. Therefore, after stage 15, the boundaries of the primitive laryngopharynx become the arytenoid swellings (cephalad) and the infraglottis (caudad). The primitive laryngopharynx becomes temporarily obliterated during development by the epithelial lamina, starting in stage 15; however, on its recanalization in stage 19, it forms the supraglottis.

STAGE 11 (17-21 SOMITES, E9.5, FIG 2)

Characteristic Features.

1. The respiratory primordium is identifiable as an epithelial thickening along the median ventral aspect of the foregut.

2. The hepatic primordium is just caudal to the respiratory primordium. Because the hepatic primordium appears as a diverticulum from the foregut (not seen in Fig 2), it is probably developmentally advanced compared to the respiratory primordium at this stage. The septum transversum is a thick plate of mesodermal tissue that separates the hepatic primordium and respiratory primordium and eventually forms the central tendon of the diaphragm. A black arrow in Fig 2 indicates the precursor region of the fourth pharyngeal pouch. Bifurcation of the dorsal aorta is seen in relation to the developing laryngopharyngeal region.

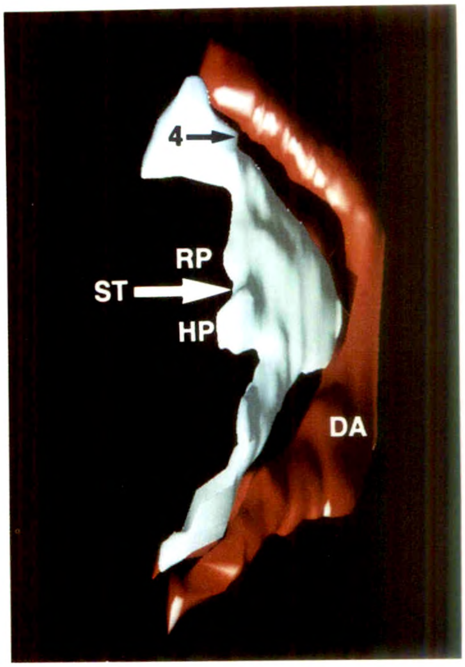

Fig 2. Stage 11 (17-somite mouse embryo, E9.5). Lateral view of 3-D reconstruction of epithelial lining of foregut. First evidence of respiratory system is indicated by epithelial thickening along ventral aspect of foregut called respiratory primordium (RP). Respiratory primordium is separated from hepatic primordium (HP) by septum transversum (ST), which is indicated by solid white arrow. Septum transversum will eventually develop into central tendon of developing diaphragm. 4 — site of developing fourth pharyngeal pouch, DA — dorsal aorta.

STAGE 12 (22-27 SOMITES, E10, FIG 3)

Characteristic Features.

1. The respiratory diverticulum results from the extension of the foregut lumen into the respiratory primordium. Figure 3A is a schematic representation of a lateral view of the laryngopharyngeal region that is sectioned in a midsagittal plane. The respiratory diverticulum is seen as an outpocketing of the foregut lumen into the respiratory primordium. The cephalic end of the respiratory diverticulum is called the primitive pharyngeal floor. The fourth pharyngeal pouch is at the same level as the pharyngeal floor.

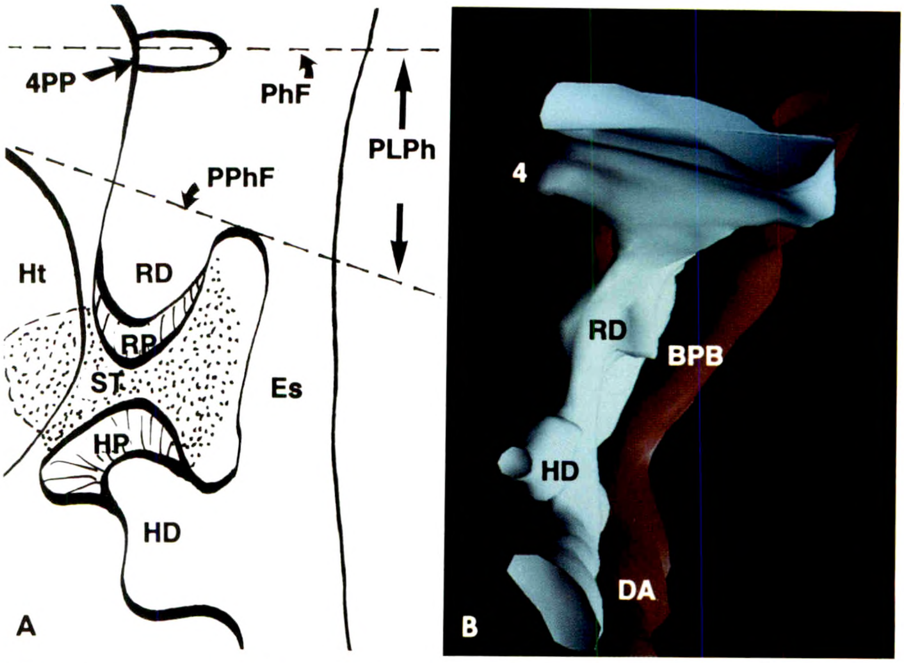

Fig 3. Stage 12 (22-somite mouse embryo, E10). A) Schematic representation of midsagittal section through laryngopharyngeal region. Respiratory diverticulum (RD) is ventral outpocketing of foregut lumen that extends into respiratory primordium (RP). Similarly, hepatic diverticulum (HD) results from extension of foregut lumen into hepatic primordium (HP). Cephalic portion of RD will eventually develop into infraglottic region of adult larynx. Site of origin of RD is called primitive pharyngeal floor (PPhF); it will eventually develop into glottic region of adult larynx. Esophagus (Es) separates from RD at level of PPhF. Primitive pharyngeal floor is separated from fourth pharyngeal pouch (4PP) by segment of foregut called primitive laryngopharynx (PLPh). Pharyngeal floor (PhF) is at same level as 4PP. Primitive laryngopharynx will eventually develop into supraglottic region of adult larynx. Ht — heart, ST — septum transversum. B) Ventral view of 3-D reconstruction of epithelial lining of laryngopharyngeal region. Respiratory diverticulum has given rise to bilateral projections called bronchopulmonary buds (BPB); they will eventually develop into lung parenchyma. DA — dorsal aorta, 4 — fourth pharyngeal pouch.

2. The fourth pharyngeal pouch is well developed as a lateral outpocketing of the foregut lumen (Fig 3B). The fourth pharyngeal pouch represents the level of the pharyngeal floor (Fig 3A).

3. The primitive laryngopharynx is the segment of foregut that lies between the fourth pharyngeal pouch as its cephalic border, and the primitive pharyngeal floor as its caudal border. The primitive laryngopharynx will eventually develop into the adult laryngeal vestibule, or supraglottic region.

4. Later in this stage, bronchopulmonary buds appear from the lateral aspect of the respiratory diverticulum (Fig 3B; a ventral view of the foregut region).

5. The distance between the respiratory primordium and the hepatic diverticulum (Fig 3B) has increased since stage 11. Because of the differential growths of the rapidly enlarging liver and heart against the septum transversum, the foregut segment between the respiratory and hepatic anlagen is lengthened. Specifically, the hepatic primordium enlarges along the caudal aspect of the septum transversum, whereas the developing heart is anchored to, and enlarges along, its cephalic aspect; both contribute to the caudocranial elongation of the foregut (Fig 3A).

STAGE 13 (28-33 SOMITES, E10.5, FIG 4)

Characteristic Features.

1. Two primary main stem bronchi (solid curved white arrow in Fig 4A) appear as a result of the continued dorsocaudal elongation of the bronchopulmonary buds. In addition to the developing heart, the bronchopulmonary buds are also tethered to the cephalic aspect of the septum transversum, and eventually will develop into the lung parenchyma.

2. The carina, which represents the location of the bifurcation of the main stem bronchi, originates from the caudal aspect of the respiratory diverticulum. With the continued caudal descent of the bronchopulmonary buds, the carina appears as a separate and distinct region from the respiratory diverticulum.

3. The foregut segment between the fourth pharyngeal pouch and the origin of the respiratory diverticulum becomes compressed bilaterally and elongated dorsoventrally, forming the so-called primitive laryngopharynx (open white arrow in Fig 4A).

STAGE 14 (34-36 SOMITES, E11)

Formation of Laryngeal Vestibule (Figs 5 and 6).

1. The rima glottidis develops from the entrance

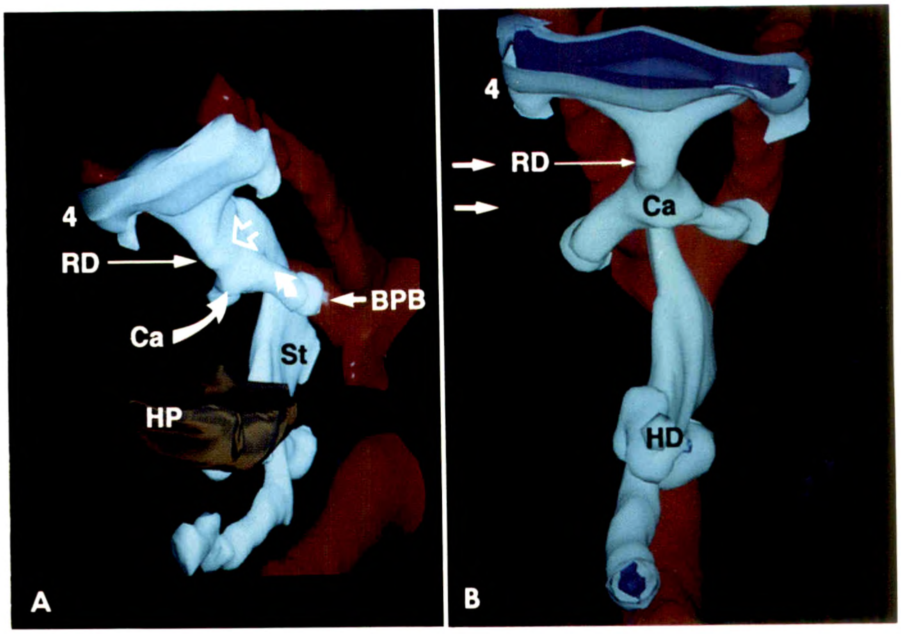

Fig 4. Stage 13 (28-somite mouse embryo, E10.5). A) Ventrolateral view of epithelial lining of foregut. Bronchopulmonary buds (BPB) have continued to be drawn dorsocaudally from respiratory diverticulum (RD), due to cephalic rotation of embryo and because BPB are tethered to septum transversum, to give rise to primary main stem bronchi (curved solid white arrow). Site of origin of BPB is called carina (Ca). Carina develops from caudal aspect of RD. Foregut lumen between fourth pharyngeal pouch (4) and RD is called primitive laryngopharynx (open white arrow). Primitive laryngopharynx becomes compressed bilaterally. St — stomach, HP — hepatic primordium. B) Ventral view of epithelial lining of foregut. Carina is seen as separate and distinct region originating from caudal aspect of RD. Two white solid arrows indicate distance between RD and Ca. HD — hepatic diverticulum.

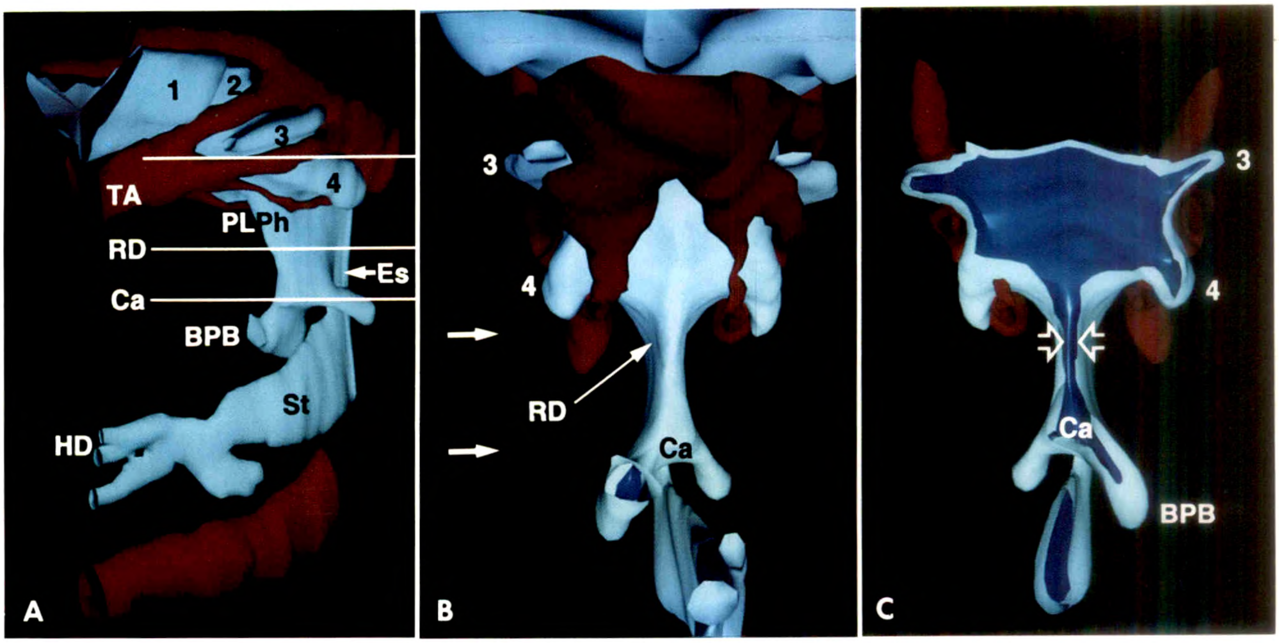

Fig 5. Stage 14 (35-somite mouse embryo, E11). A) Lateral view of 3-D reconstruction of epithelial lining of foregut with its associated branchial arch arteries. This reconstruction should be compared with 28-somite mouse embryo seen in Fig 4A to identify following anatomic changes: primitive laryngopharynx (PLPh) continues to lengthen and become compressed bilaterally; carina (Ca) continues to descend from site of respiratory diverticulum (RD); and esophagus (Es) becomes stretched between PLPh and stomach (St) to give rise to thin, circular lumen. TA — thyroarytenoid anlage, BPB — bronchopulmonary buds, HD — hepatic diverticulum. B) Ventral view of 3-D reconstruction of epithelial lining of laryngopharyngeal region. Compared with Fig 4B, lengthening of Ca from RD is demonstrated with two white solid arrows. Lengthening of this foregut segment will eventually give rise, in part, to developing trachea. C) Coronal section of embryo shown in B, showing internal lumen (dark blue). Open white arrows indicate where bilateral compression of PLPh lumen will occur in subsequent stages. Numerals 1 to 4 — first to fourth pharyngeal pouches.

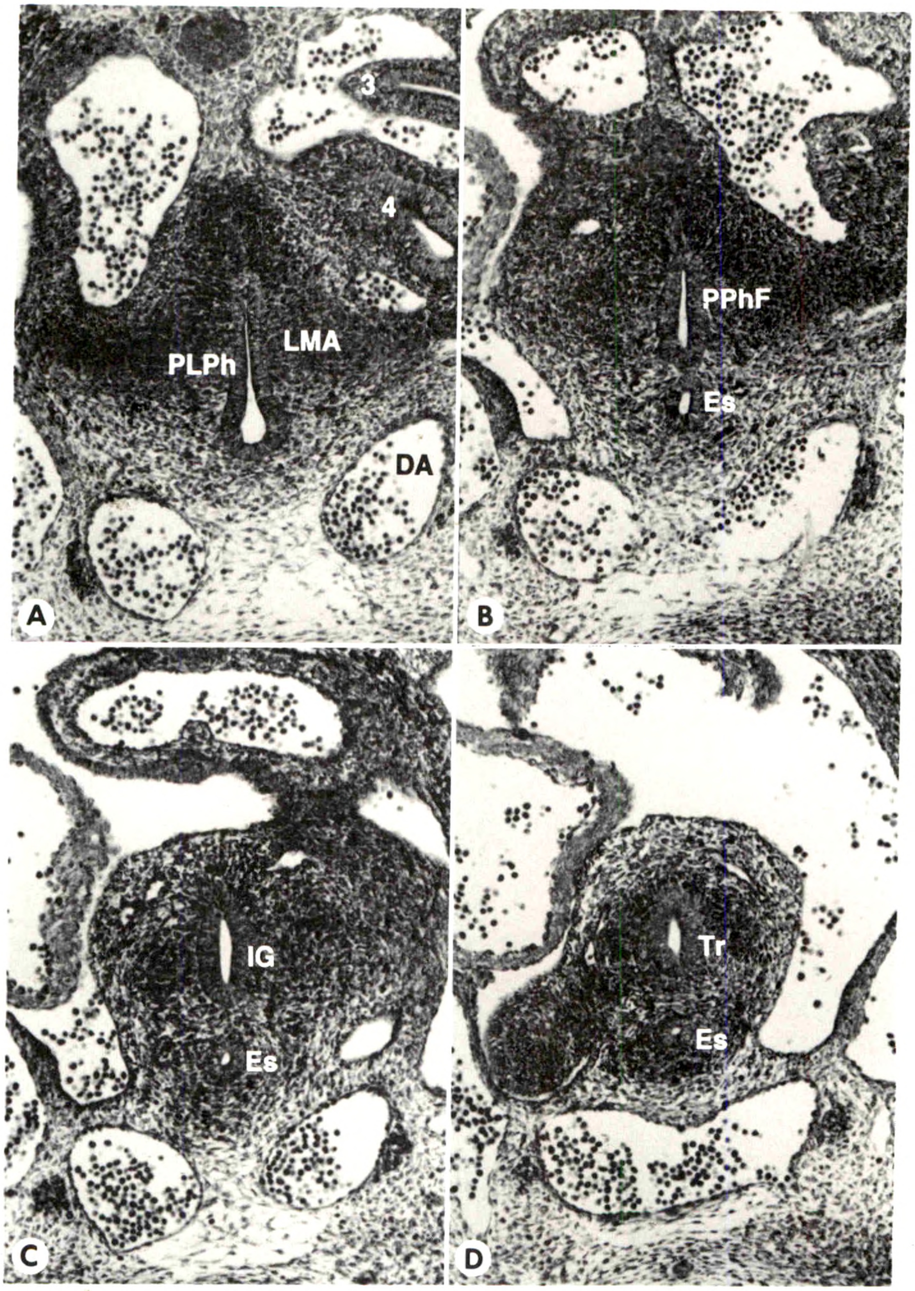

Fig 6. Stage 14 (36-somite mouse embryo, E11.5). A-D) Series of axial histologic sections taken through developing laryngopharyngeal region is demonstrated in craniocaudal sequence. A) Section taken at level of fourth pharyngeal pouch. This represents site of origin of primitive laryngopharynx (PLPh). Laryngeal mesodermal anlagen (LMA) are seen as bilateral triangular condensations flanking ventral lumen of PLPh. DA — dorsal aorta. B) Section taken at level of primitive pharyngeal floor (PPhF). Slitlike shape of this lumen will eventually give rise to glottic region. This represents most caudal region of PLPh, and occurs at site of bifurcation of infraglottis (just caudal to PPhF) from esophagus (Es). C) Section taken at level of lumen of infraglottis (IG), which is now separated from Es. Lumen of IG always remains patent during normal laryngeal development. D) Section taken at level of trachea (Tr). Shape of this lumen is becoming ovoid. (Continued on next page.)

into the respiratory diverticulum or the primitive pharyngeal floor (Fig 6B).

2. The infraglottis is formed. The continued caudal descent of the carina and bronchopulmonary buds from the respiratory diverticulum is shown by comparing the distance separating the solid white arrows in Figs 4B and 5B. Coronal sectioning of the 35-somite embryo in Fig 5B is shown in Fig 5C. The lumen appears uniform from the fourth pharyngeal pouch level to the carina when sectioned in the coronal plane. Open white arrows indicate the site in which the primitive laryngopharynx will become compressed to form the epithelial lamina. In the 36-somite embryo, the upright-triangle shape of the infraglottic lumen demonstrates that the infraglottis will form just caudal to the primitive laryngopharynx

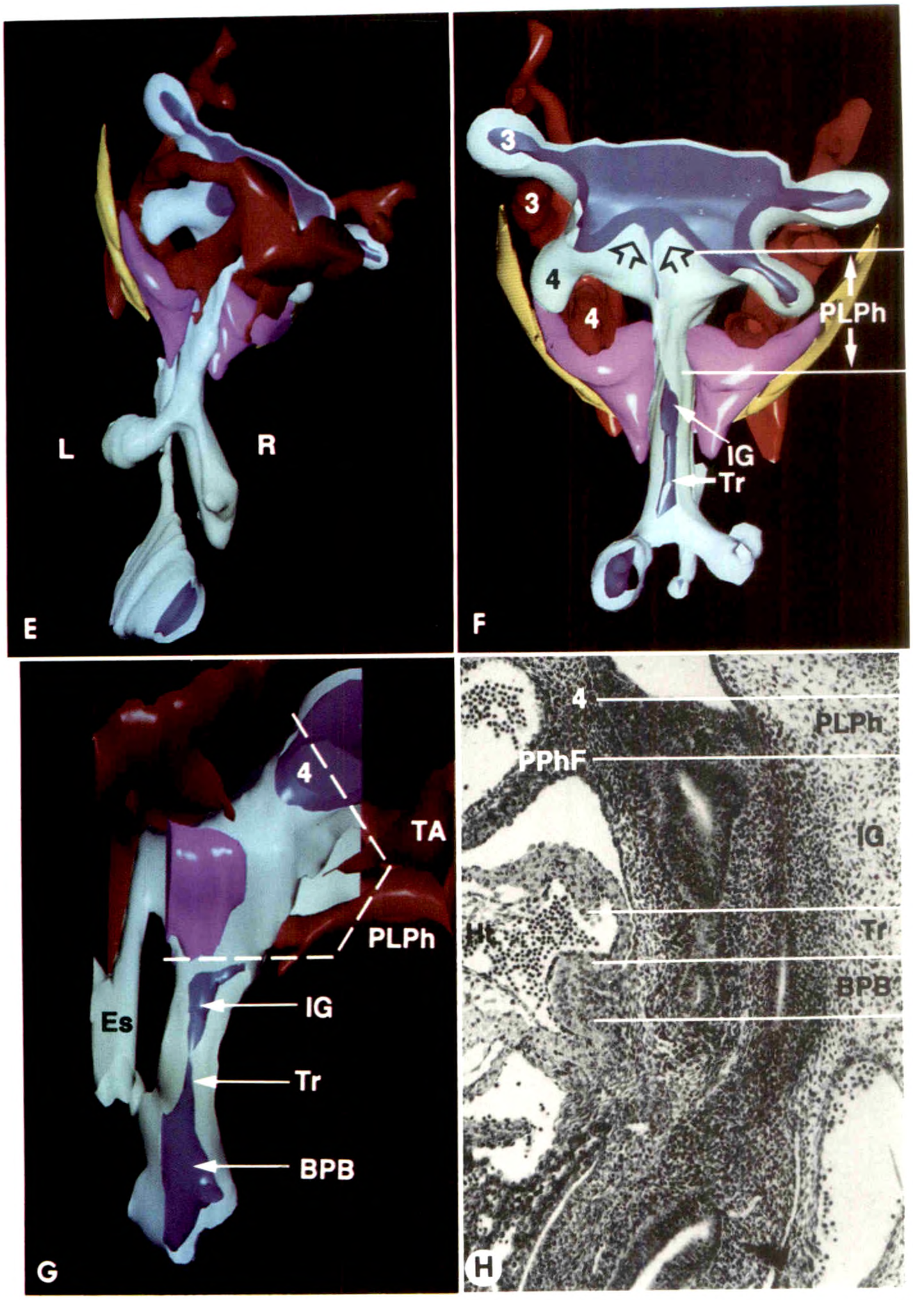

Fig 6, continued. E) Ventral view of 3-D reconstruction of embryo developed from histologic sections shown in A-D. Note trifurcation of right bronchopulmonary bud. F) Coronal section of embryo shown in E. Compared to embryo shown in Fig 5C, note following anatomic changes. Region of PLPh has become compressed bilaterally, likely because of forces exerted by LMA (purple) and fourth branchial artery (4, red). This region of compression was shown previously by open white arrows in Fig 5C. Also, infraglottis (IG) has characteristic shape of upright triangle when sectioned in this coronal plane. Infraglottis is separated from carina by ovoid-circular lumen of trachea. Also, at level of fourth pharyngeal pouch (4), elevation of median pharyngeal floor, as indicated by open black arrows, will give rise to developing arytenoid swellings. G) Midsagittal section of laryngopharyngeal region of embryo shown in E and F. Region defined by white broken lines represents PLPh, as it extends from fourth pharyngeal pouch (4) to IG. Laryngeal mesodermal anlagen (purple) are seen compressing this region. Infraglottis has characteristic inverted-triangle shape when sectioned in sagittal plane. Infraglottis is separated from bronchopulmonary buds (BPB) by developing trachea (Tr). H) Midsagittal histologic section of laryngopharyngeal region. This is mirror image of 3-D reconstruction shown in G. Note that IG lumen is patent, has inverted-triangle shape, and is separated from BPB by Tr.

(Fig 6F). A sagittal histologic section of the same staged embryo (Fig 6G,H) shows that the lumen just inferior to the primitive laryngopharynx has the shape of an inverted triangle. The upright-triangle and inverted-triangle shape of the infraglottic lumen resembles that of the adult laryngeal cavity just caudal to the rima glottidis, ie, the infraglottis, or conus elasticus. Therefore, the infraglottic lumen initially demonstrates its characteristic shape when viewed from both a coronal and a midsagittal plane (36-

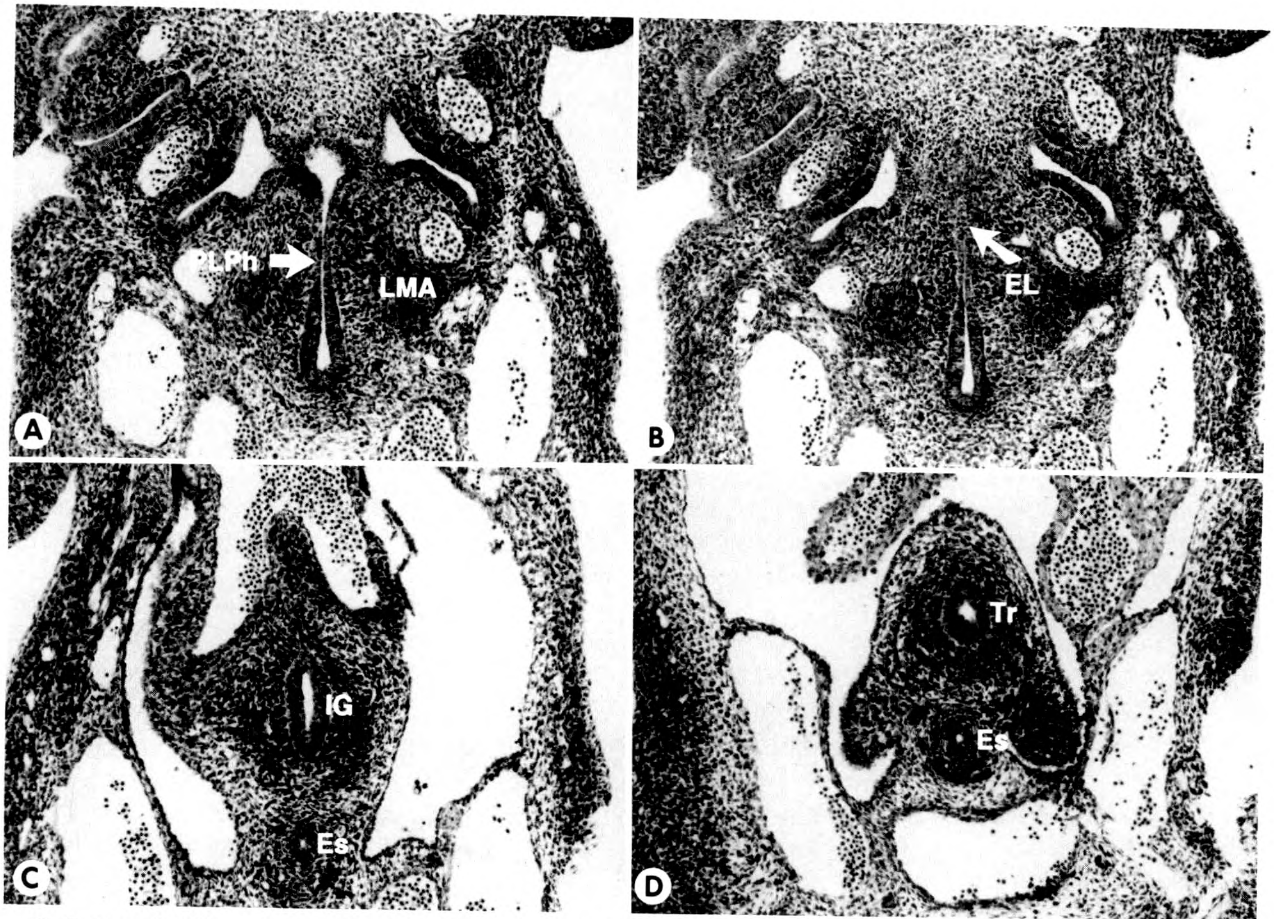

Fig 7. Stage 15 (44-somite mouse embryo, E11.5 to E12). A-D) Series of axial histologic sections taken through developing laryngopharyngeal region is demonstrated in craniocaudal sequence. A) Level of this section is taken at entrance to primitive laryngopharynx (PLPh), which is bounded bilaterally by laryngeal mesodermal anlagen (LMA). B) Section taken through center of PLPh. Ventral aspect of lumen has become obliterated (solid white arrow) due to merging of lateral pharyngeal walls. This is called epithelial lamina (EL). C) Section taken at level of completely patent infraglottic lumen (IG). D) Section taken at level of trachea (Tr). Es — esophagus.

somite embryo).

3. The trachea is formed. The tracheal lumen is uniformly circular, and results from the continued caudal descent of the carina and bronchopulmonary buds from the infraglottis.

4. The arytenoid swellings are formed. The close proximity of the expanding fourth branchial artery to the entrance of the primitive laryngopharynx is seen in Fig 6E,F. In addition, the laryngeal mesodermal anlagen (purple) are also proliferating rapidly in the primitive laryngopharynx region. It is the combination of these two forces that contributes to the formation of the arytenoid swellings; bilateral longitudinal ridges that flank the median slit in the pharyngeal floor lead into the primitive laryngopharynx. The forces giving rise to the arytenoid swellings are represented by open black arrows in Fig 6F.

5. The region of the primitive laryngopharynx is demarcated by horizontal white lines in Fig 6F. The cephalic border is at the level of the fourth pharyngeal pouch, or at the region of the developing arytenoid swellings (represented by black open arrows in Fig 6F). The caudal border is at the level of the glottis, or the cephalic portion of the infraglottis (seen as an upright triangle in this coronal section). The primitive laryngopharynx continues to become compressed by the enlarging laryngeal mesodermal anlagen (purple). A ridge is forming along the ventral aspect of the primitive laryngopharynx by the compressive force of the laryngeal mesodermal anlagen.

6. The main stem bronchi divide. In Fig 5E, the right main stem trifurcates, whereas the left main stem bifurcates (not seen in this view).

Formation of Laryngeal Skeleton and Musculature. On either side of the primitive laryngopharynx is a wedge-shape condensation of mesoderm that will give rise to the laryngeal cartilages and musculature. These are called the laryngeal mesodermal anlagen and they are first seen in the 36-somite mouse embryo (Fig 6A,E-G).

STAGE 15 (37-44 SOMITES, E11.5-E12, FIG 7)

The characteristic feature in the formation of the laryngeal vestibule is the formation of the epithelial lamina (Fig 7). Because of the same forces that give rise to the arytenoid swellings, the ventral aspect of the primitive laryngopharynx lumen becomes compressed by the convergence of its lateral epithelial walls. This is called the epithelial lamina.

STAGE 16 (45-51 SOMITES, E12-E12.5, FIG 8)

Formation of Laryngeal Vestibule.

1. The epithelial lamina, which started along the ventral aspect of the primitive laryngopharynx, has nearly obliterated the primitive laryngopharynx except for a narrow patent pharyngoglottic duct dorsally (Fig 8C-E,I,J). The pharyngoglottic duct connects the hypopharynx (the lumen above the arytenoid swellings) to the infraglottis. Since the glottic region is just cephalic to the infraglottic lumen (which remains patent during normal laryngeal development), the epithelial lamina represents the normal developmental obliteration of the supraglottic larynx (Fig 6A,B,D).

2. The laryngeal cecum develops along the ventrocephalic aspect of the epithelial lamina. It is a transverse blind pouch that forms between the arytenoid swellings dorsally, and the hypobranchial eminence ventrally (Fig 8B-D,I-K). It eventually will descend to the glottic level. In this stage, it descends caudally from one third to one half the cephalic length of the primitive laryngopharynx.

3. The cephalic aspects of the arytenoid swellings (Fig 8A,B and yellow in Fig 8I) appear as conical bulges that arise from the pharyngeal floor. They flank the entrance into the primitive laryngopharynx (Fig 8L) and they will replace the fourth pharyngeal pouch as the superior limit of the primitive laryngopharynx.

4. The infraglottis demonstrates its classic inverted-triangle appearance on sagittal sectioning in Fig 8I,K. The glottis is just cephalad to the infraglottic region (Fig 8I,K). The infraglottis has replaced the primitive pharyngeal floor as the inferior limit of the primitive laryngopharynx.

Formation of Laryngeal Skeleton and Musculature. The laryngeal mesodermal anlagen have consolidated into two distinct masses; one resembles the hyoid anlage (Fig 8H,L,M), with the presence of both greater and lesser wings (Fig 8L). The other appears as the cricothyroid anlage complex. The U-shape thyroid (Fig 8D) anlage gives rise inferiorly to three distinct regions that form the cricoid anlage: one ventromedian and two dorsolateral (Fig 8E,M). Figure 8L is a dorsal view of the laryngeal mesodermal anlagen. The dorsal cleft of the cricothyroid anlage (curved white solid arrow in Fig 8L) in this view suggests that posterior laryngeal cleft malformations may occur secondary to cessation of laryngeal development at this or at subsequent stages of development up to stage 19.

STAGES 17 AND 18 (52-64 SOMITES, E13-14, FIG 9)

Formation of Laryngeal Vestibule.

1. Similar to stage 16, the epithelial lamina still obliterates the primitive laryngopharynx except for the laryngeal cecum ventrally and the pharyngoglottic duct dorsally (Fig 9A-C).

2. The laryngeal cecum continues to descend in the E13 embryo (Fig 9A,C), reaching its caudal limit at the level of the glottis in the E14 embryo (see G in Fig 9A,C). The site of origin of the laryngeal cecum is between the dorsal arytenoid swellings (yellow) and the ventral epiglottis (green) in Fig 9E (curved solid white arrow).

3. The epiglottis appears as a swelling emanating from the dorsal aspect of the hypobranchial eminence (green in Figs 8L and 9E,H). Note that the arytenoid swellings have continued to enlarge (yellow in Fig 9E,H).

4. The infraglottis is identified by its inverted-triangle appearance on sagittal sectioning (Fig 9A,C).

Formation of Laryngeal Skeleton and Musculature.

1. Chondrification centers of the laryngeal mesodermal anlagen first appear along the ventral aspect of the hyoid anlage in E13 (Fig 9D,F), and along the ventral aspect of the cricoid anlage in E14 (Fig 9G).

2. Lateral and dorsal fusion of the cricoid anlage occurs in E14. Lateral fusion of the cricoid cartilage anlage is demonstrated by comparing Fig 9D and 9G. Dorsal fusion is seen by comparing the curved solid white arrows from Fig 8L (E12) to Fig 9F (E13) to Fig 9H (E14), where the median groove along the dorsal aspect of the cricoid anlage is a remnant indicating fusion has occurred. Incomplete fusion can lead to the different degrees of posterior laryngotracheoesophageal clefting seen clinically.

STAGES 19 THROUGH 23 (E15-16, FIGS 10 AND 11)

Formation of Laryngeal Vestibule.

1. The epithelial lamina is recanalized, as indicated by vesicles appearing between the lateral walls of the primitive laryngopharynx (bold black arrow in Fig 10A,B). Eventually, the pharyngoglottic duct will be brought into continuity with the laryngeal cecum to

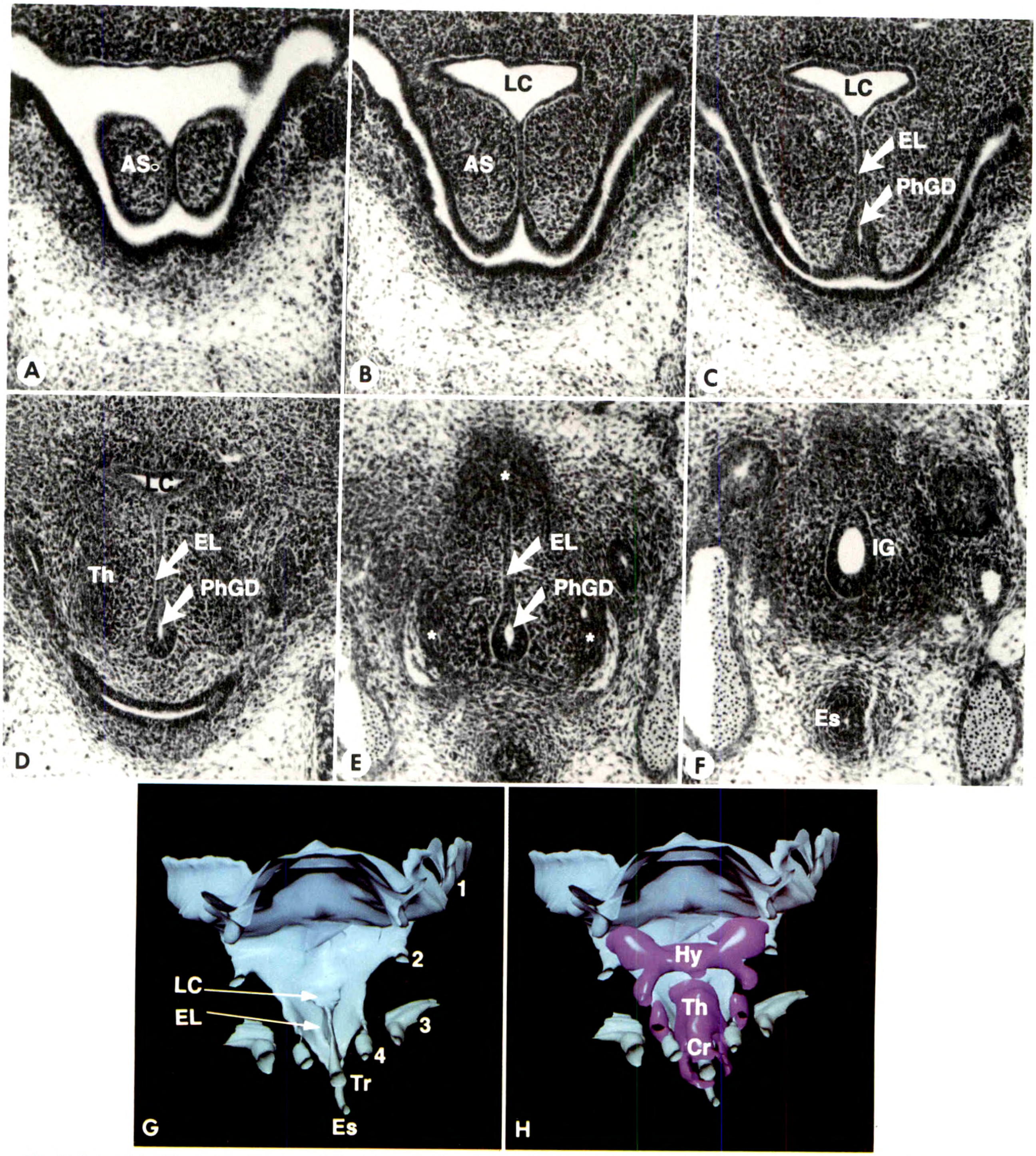

Fig 8. Stage 16 (50-somite mouse embryo, E12 to E12.5). A-F) Series of axial histologic sections taken through developing laryngopharyngeal region is demonstrated in craniocaudal sequence. Sections C-E are sequential sections taken through primitive laryngopharynx. A) Section taken at level of hypopharynx, just above entrance to primitive laryngopharynx. Arytenoid swellings (AS) are bilateral conical elevations of median pharyngeal floor that bound entrance to primitive laryngopharynx. B) As sections progress caudally, triangular lumen appears along ventral aspect of AS, called laryngeal cecum (LC). C) Section taken at level of primitive laryngopharynx. Epithelial lamina (EL) has completely obliterated lumen of primitive laryngopharynx except for LC, which appears along ventral aspect of EL. In addition, narrowly patent pharyngoglottic duct (PhGD) appears along dorsal aspect of EL. D) Laryngeal cecum is tapered in its lateral and caudal extent. Pharyngoglottic duct continues caudally, along dorsal aspect of EL, until it reaches infraglottic region, seen in F. A portion of laryngeal mesodermal anlage has consolidated into U-shape region, indicating thyroid anlage (Th). E) Section taken at most caudal region of primitive laryngopharynx. Thyroid cartilage anlage has consolidated into three distinct regions (asterisks) at level of cricoid anlage: two posterolateral, and one ventromedian. F) Section taken at level of infraglottis (IG). Es — esophagus. G) 3-D reconstruction of epithelial lining of laryngopharyngeal region (derived from histologic sequence of A-F) viewed from ventral perspective. H) Laryngeal mesodermal anlagen of hyoid (Hy), thyroid (Th), and cricoid (Cr; all purple) are superimposed on image seen in G. (Continued on next page.)

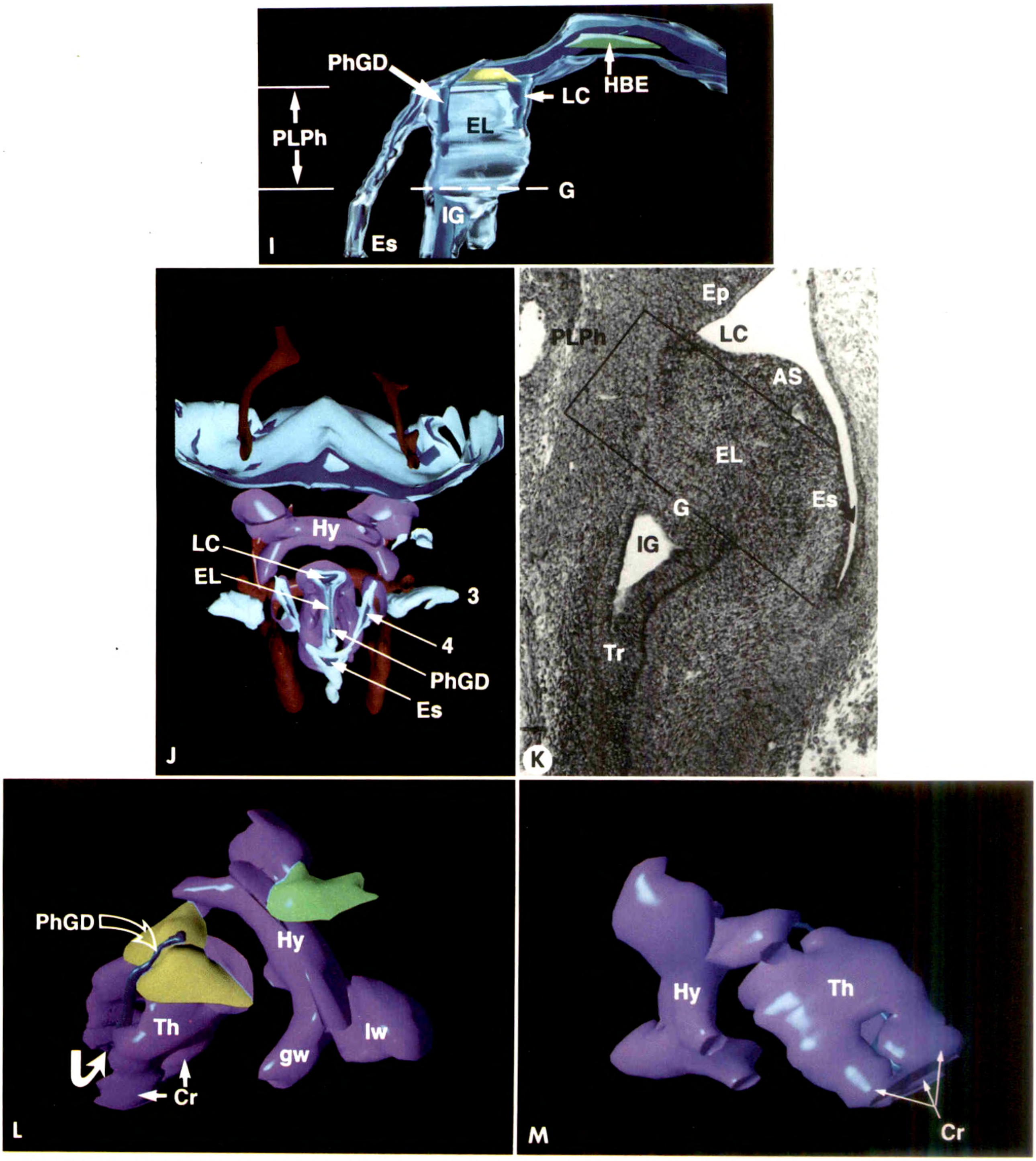

Fig 8, continued. I) Lateral view of 3-D reconstruction of foregut epithelial lining. Glass simulation was used so that internal (blue) luminal anatomy could be visualized. Primitive laryngopharynx (PLPh) extends from floor of pharynx (level of AS; yellow) to infraglottic region below (IG; previously cephalic end of respiratory diverticulum). Glottis (G) is just cephalic to IG. Primitive laryngopharynx is completely obliterated except for narrow PhGD dorsally and developing LC ventrally. J) Axial section of I. Anatomic findings correlate with those seen on histologic section in D. K) Midsagittal histologic section that is mirror image of 3-D reconstruction seen in I. Lumen of IG has shape of inverted triangle. Glottis region forms just cephalic to IG. Foregut segment that separates IG from base of AS is primitive laryngopharynx (PLPh), which is depicted by black solid lines. Epithelial lamina represents obliterated PLPh. Laryngeal cecum forms between AS and epiglottis (Ep). L) Dorsosuperolateral view of 3-D reconstruction of laryngeal mesodermal anlagen (purple) and AS (yellow). Curved solid white arrow demonstrates dorsal cleft along cricothyroid anlage (which corresponds to course of PhGD). lw — lesser wing of hyoid anlage or cartilage, gw — greater wing of hyoid anlage or cartilage. M) Lateral view of 3-D reconstruction of laryngeal mesodermal anlagen (purple). Cricoid anlage appears to originate from three distinct regions: two posterolateral and one ventromedian. Numerals 1 to 4 — first to fourth pharyngeal pouches.

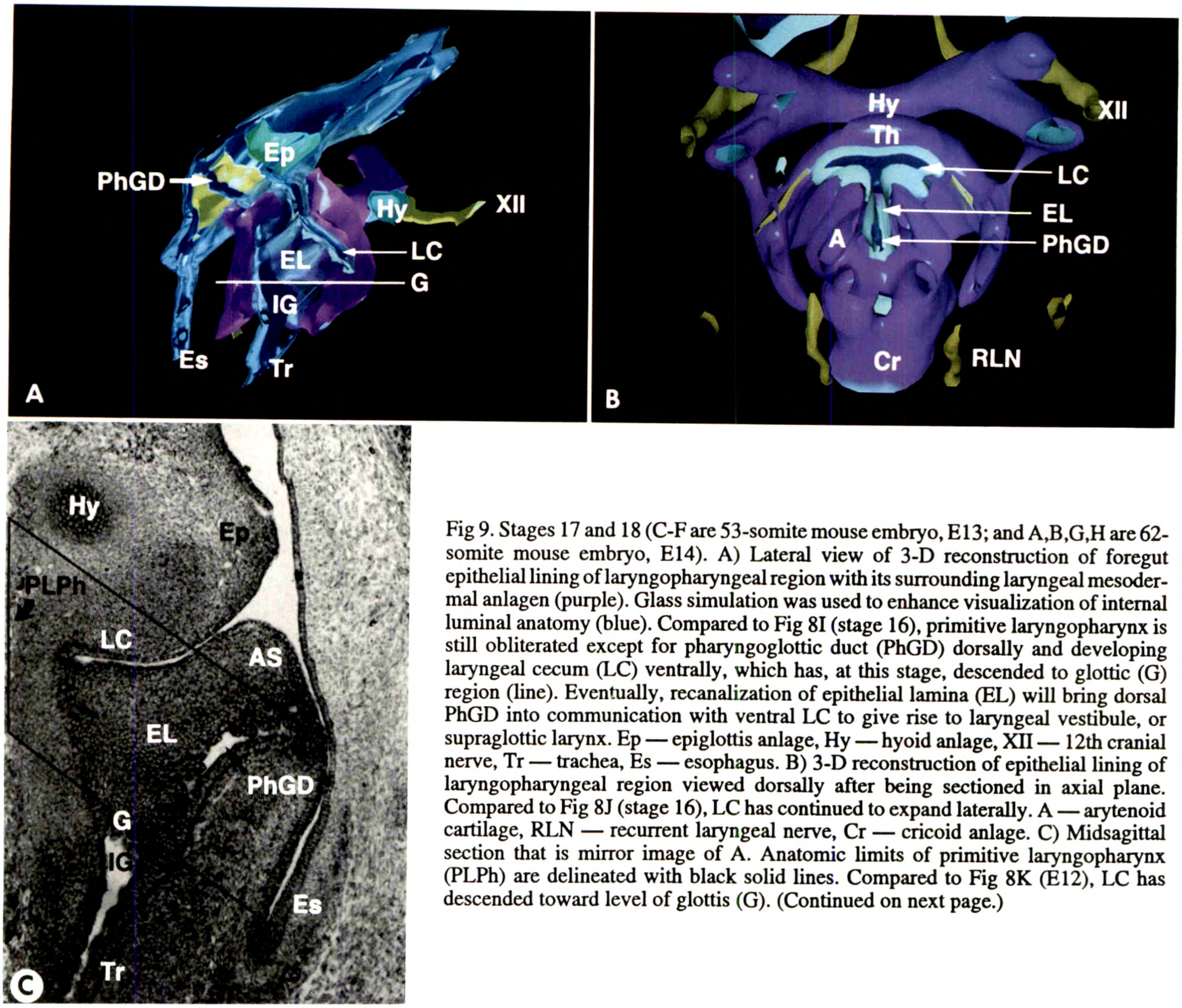

Fig 9. Stages 17 and 18 (C-F are 53-somite mouse embryo, E13; and A,B,G,H are 62-somite mouse embryo, E14). A) Lateral view of 3-D reconstruction of foregut epithelial lining of laryngopharyngeal region with its surrounding laryngeal mesodermal anlagen (purple). Glass simulation was used to enhance visualization of internal luminal anatomy (blue). Compared to Fig 8I (stage 16), primitive laryngopharynx is still obliterated except for pharyngoglottic duct (PhGD) dorsally and developing laryngeal cecum (LC) ventrally, which has, at this stage, descended to glottic (G) region (line). Eventually, recanalization of epithelial lamina (EL) will bring dorsal PhGD into communication with ventral LC to give rise to laryngeal vestibule, or supraglottic larynx. Ep — epiglottis anlage, Hy — hyoid anlage, XII — 12th cranial nerve, Tr — trachea, Es — esophagus. B) 3-D reconstruction of epithelial lining of laryngopharyngeal region viewed dorsally after being sectioned in axial plane. Compared to Fig 8J (stage 16), LC has continued to expand laterally. A — arytenoid cartilage, RLN — recurrent laryngeal nerve, Cr — cricoid anlage. C) Midsagittal section that is mirror image of A. Anatomic limits of primitive laryngopharynx (PLPh) are delineated with black solid lines. Compared to Fig 8K (E12), LC has descended toward level of glottis (G). (Continued on next page.)

form the laryngeal vestibule, or supraglottis. It is first seen in the E15 embryo (Fig 10A,B), and the process starts along the cephalodorsal aspect of the epithelial lamina and progresses ventrocaudally. Figure 10B is a coronal section that demonstrates the recanalization of the epithelial lamina at the level of the arytenoids. Figure 10C is an axial section that shows that the epithelial lamina still obliterates the lumen at the glottic level, except for the pharyngoglottic duct along the epithelial lamina's dorsal border. Figure 11B is a histologic coronal section of an E16 embryo at the level of the free vocal cords. This section shows that recanalization is not complete ventrally. Figure 11C is a 3-D reconstruction of the E16 mouse laryngopharynx. The white broken lines indicate where recanalization has not occurred. This is the same location in which glottic webs may occur.

2. Ventricular outgrowths appear from the lateral aspect of the laryngeal cecum (Fig 11H). The lateral extent of the ventricular outgrowths is not as developed as in human embryos (which extend laterally to the thyroid cartilage). The ventricular outgrowths will form the laryngeal ventricles in the adult larynx.

3. The infraglottic lumen continues to enlarge in all dimensions in E16 (Fig 11A,B).

Formation of Laryngeal Skeleton and Musculature.

1. There is complete chondrification of laryngeal cartilages (Figs 10C and 11D,E,G). In Fig 10A (E15), the epiglottis appears to be precartilaginous; however, chondrification of the remaining laryngeal cartilages is complete. At age E16, chondrification of the epiglottis is complete (Fig 11E,G). There is incomplete fusion of the tracheal rings, and the cuneiform and corniculate cartilages are fused with the apex of the arytenoid in a normal mature mouse larynx (Fig 11E).

2. The myofibrils for the muscles of the intrinsic larynx are well developed by E15. Figure 10C is an

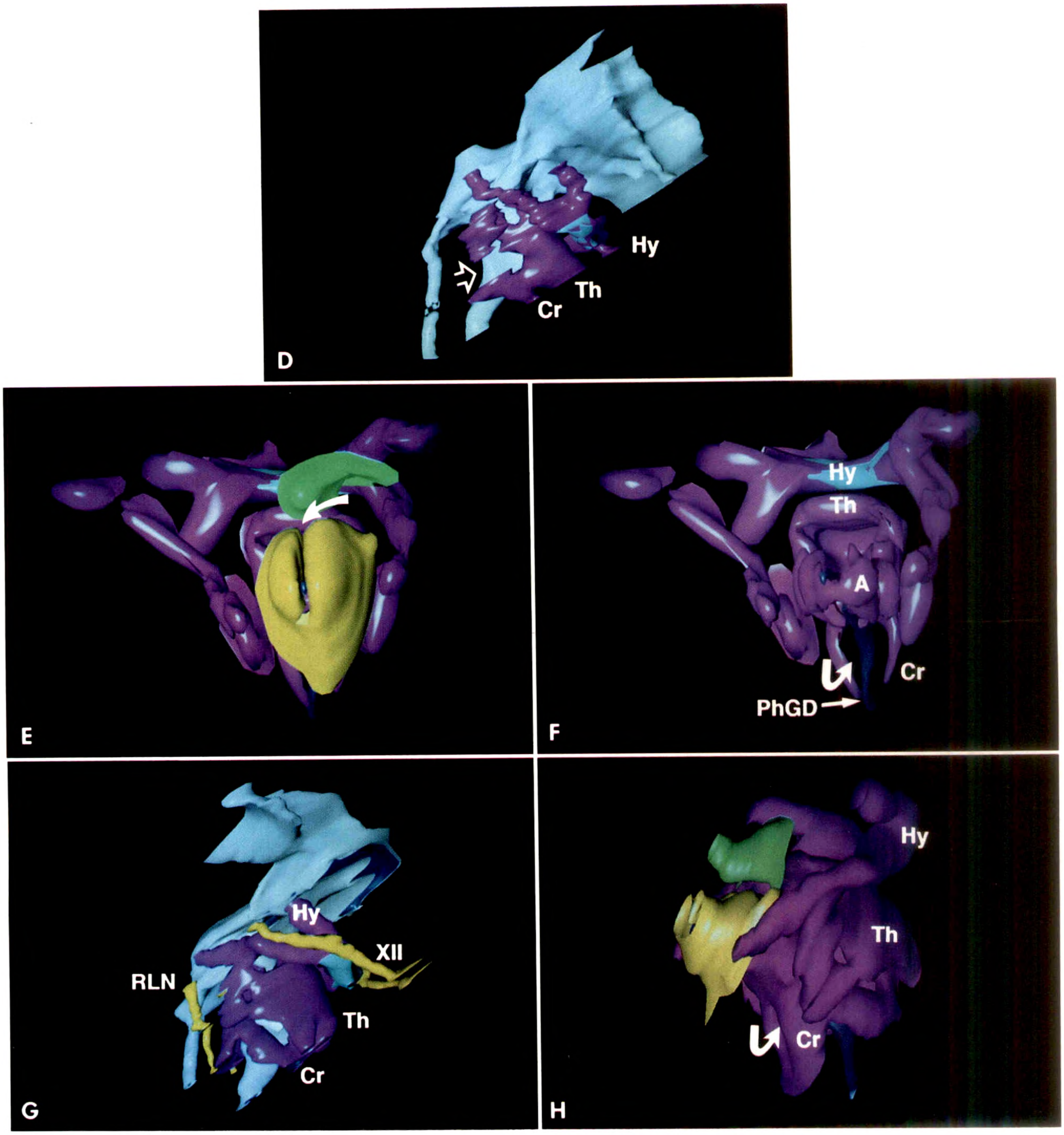

Fig 9, continued. D) Lateral view of laryngeal mesodermal anlagen (purple) superimposed on epithelial lining of laryngopharyngeal region (E13). Cricoid anlage (Cr) still appears as three distinct regions. Notice that chondrification center (light blue) first appears along ventral aspect of Hy. Fusion of cricoid anlage laterally has not yet occurred (open white arrow). E) Dorsal view of arytenoid swellings (yellow) and epiglottis (green) superimposed on laryngeal mesodermal anlagen seen in G. Curved white solid arrow indicates site at which LC originates prior to its descent along ventral aspect of EL. F) Dorsal view of laryngeal mesodermal anlagen (E13). Fusion of mesenchyme begins at arytenoid region; however, it is incomplete caudally (curved white solid arrow). G) Lateral view of laryngeal mesodermal anlagen (purple) superimposed on epithelial lining of laryngopharyngeal region (E14). Fusion of Cr has occurred laterally. Chondrification center appears along ventral aspect of Cr, and chondrification of Hy advances dorsally compared to D. H) Dorsal view of laryngeal mesodermal anlagen (E14). Site of dorsal fusion of mesenchyme is demonstrated by vertical line along dorsal cricoid lamina (indicated by solid white curved arrow). It has been suggested that it is incomplete dorsal fusion of Cr that can give rise to full spectrum of laryngotracheal clefts seen clinically.

axial section through an E15 mouse larynx at the glottic level. The thyroarytenoid and vocalis muscles are distinguishable.

FETAL STAGE (FIG 12)

Formation of Laryngeal Vestibule. The epithelial lamina has completely recanalized, bringing the

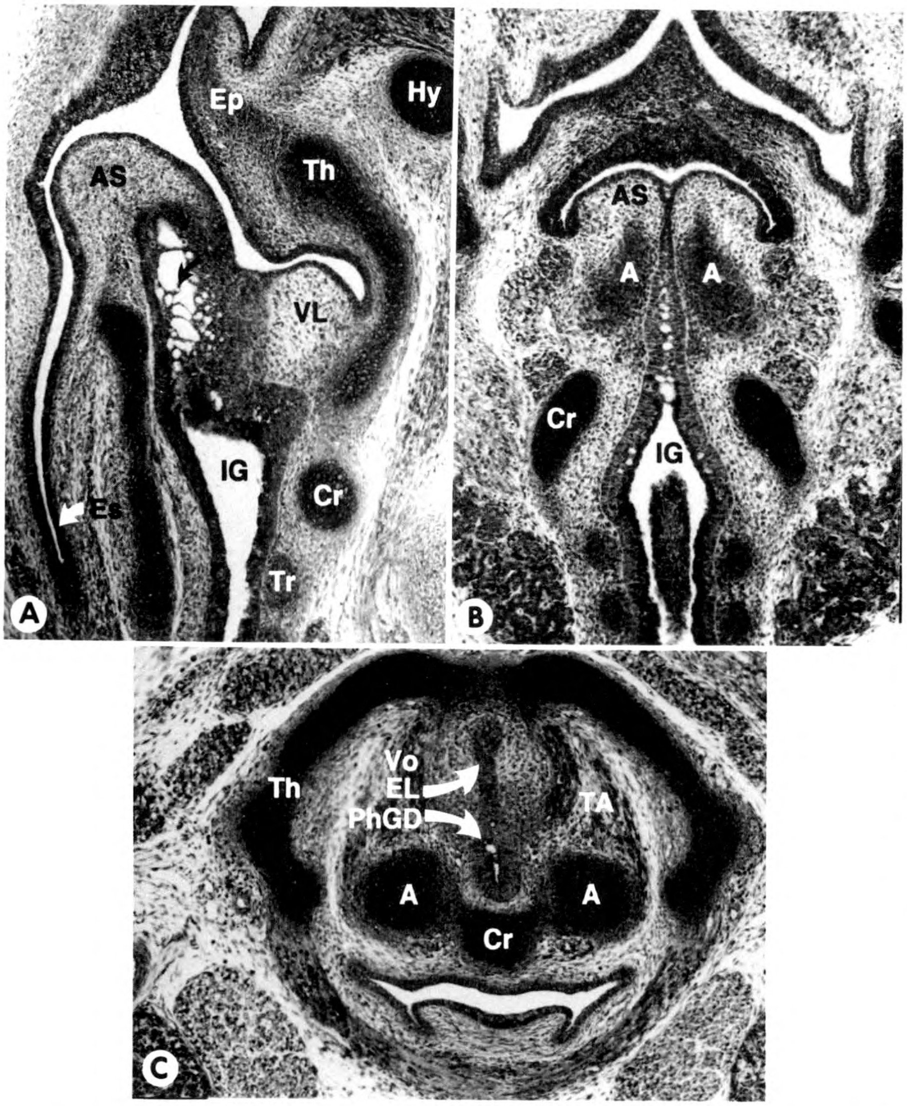

Fig 10. Stages 19 and 20 (E15 mouse embryo). A) Midsagittal section through laryngopharyngeal region as vesicles are seen along dorsocephalic aspect of epithelial lamina. Recanalization of epithelial lamina progresses in ventrocaudal direction. Hence, last portion of laryngeal vestibule to recanalize is ventral glottic region. Chondrification of all laryngeal cartilages is complete except for epiglottis (Ep). Ventral aspect of glottic region has differentiated into vocal ligaments (VL). Recanalization is indicated by solid black arrow. AS — arytenoid swellings, Hy — hyoid anlage, Th — thyroid cartilage, IG — infraglottis, Es — esophagus, Cr — cricoid anlage, Tr — trachea. B) Coronal section at same plane as arytenoid cartilage (A). C) Axial section demonstrating persistence of epithelial lamina (EL) at glottic level. Pharyngoglottic duct (PhGD) is narrow patent lumen dorsal to EL. Myofibrils of thyroarytenoid (TA) and vocalis muscles (Vo) are demonstrated.

supraglottis into communication with the infraglottis via the vocal ligaments. Figure 12 demonstrates this, and the red color indicates the vocal ligaments.

DISCUSSION

In Observations, the critical stages in the development of the laryngeal vestibule, skeleton, and musculature were demonstrated with two-dimensional histologic sections and 3-D computerized solid model reconstructions. In the present section, the history of laryngeal development is described by reviewing the theories of anatomists who based their conclusions on wax models and other types of reconstructions derived from their histologic series. The embryology of the mouse larynx will be reviewed in order to compare and contrast it with the aforementioned theories and to suggest how certain congenital anomalies of the laryngotracheobronchial system may arise from developmental arrests at critical stages of its development.

REVIEW OF LITERATURE

Wilhelm His[4] stated that the foregut segment between the lung primordium and the fourth pharyngeal pouch formed the esophagus dorsally and the trachea ventrally. He believed that the foregut became divided by an ascending tracheoesophageal septum that began as a depression that he observed behind the lung primordium in his reconstructions of the early developing larynx. Several investigators studying wax plate reconstructions of the laryngopharyngeal region of the foregut supported His' theory of the origin of the laryngeal cavity and added their own novel interpretations. In accepting His' theory of an ascending tracheoesophageal septum reaching the level of the fourth pharyngeal pouches in the process of forming the trachea, they assumed that the opening in the pharyngeal floor was the cephalic end of the trachea and esophagus.

Kallius[1] believed that the entire laryngeal cavity developed from the floor of the pharynx, cephalic to

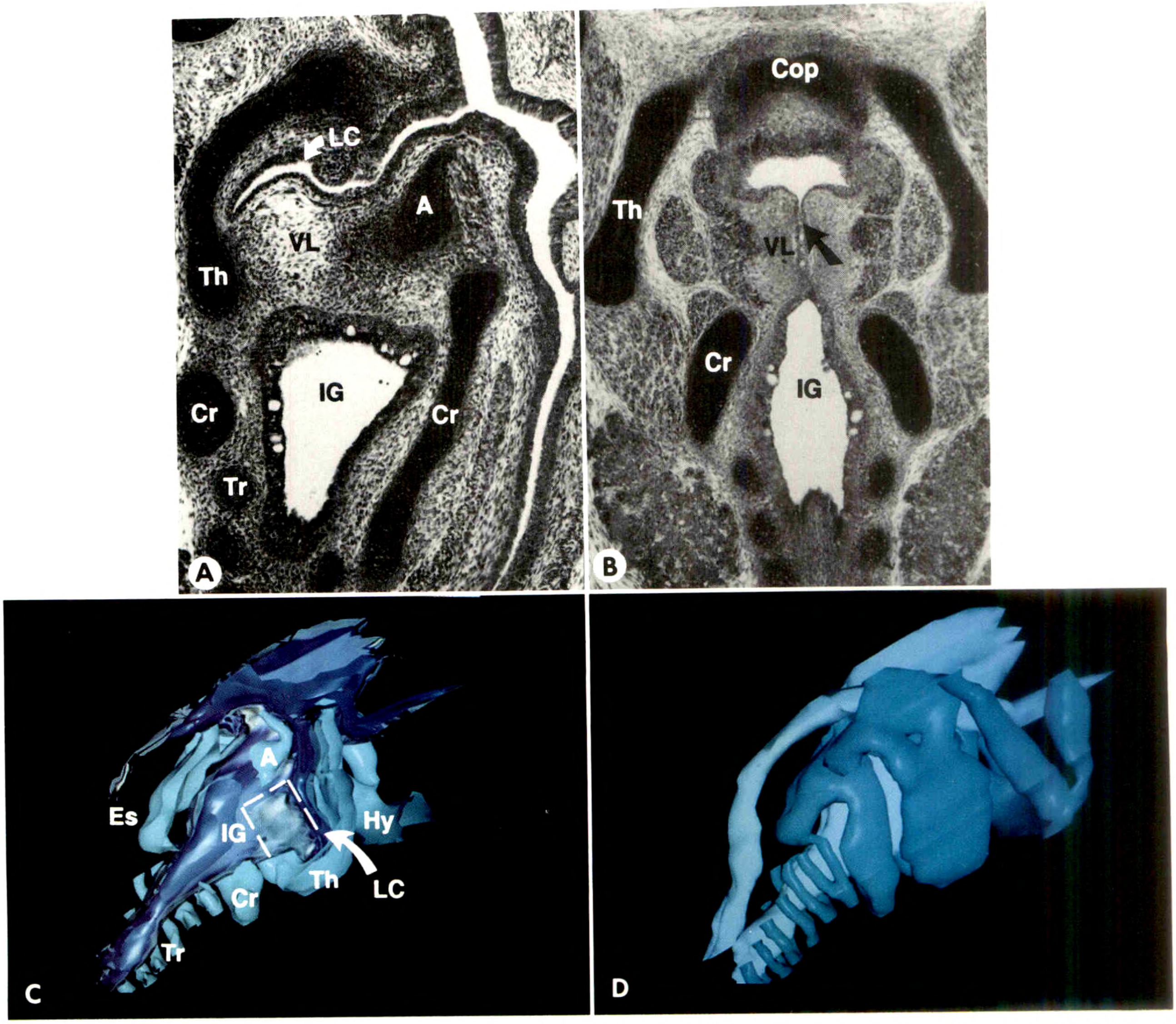

Fig 11. Stages 21 through 23 (E16 mouse embryo). A) Midsagittal section of laryngopharyngeal region. Compared to Fig 10A (stage 19), infraglottis (IG) has continued to expand. LC — laryngeal cecum, A — arytenoid cartilage, VL — vocal ligament, Th — thyroid cartilage, Cr — cricoid cartilage, Tr — trachea. B) Coronal histologic section of same-stage embryo at level of VL. Epithelial lamina (solid black arrow) has not completely recanalized at level of anterior third of glottis. Cop — copula. C) 3-D reconstruction of laryngopharyngeal region, sectioned in midsagittal plane exposing right medial aspect of embryo. Glass simulation is used to view luminal (dark blue) anatomy. White broken lines indicate area in which epithelial lamina has not completely recanalized. It has been suggested that incomplete recanalization of epithelial lamina can give rise to full spectrum of supraglottic stenosis and glottic webs seen clinically (see text). Es — esophagus. D) Lateral view of 3-D reconstruction of laryngopharyngeal epithelial lining with superimposed laryngeal cartilages. There is now complete chondrification of all laryngeal cartilages. (Continued on next page.)

the tracheal opening. More specifically, he thought bilateral conical projections (arytenoid swellings) grew up from the floor of the pharynx (level x–x in B in Fig 13[1-4,9]) on each side of the tracheal opening and fused in the median plane, obliterating most of the pharyngeal cavity to create the epithelial lamina. Consequently, he concluded that the laryngeal cecum (a potential space created along the ventral aspect of the arytenoid swellings) resulted from the passive growth of the arytenoid swellings into the pharyngeal cavity and that this cecum would eventually become the laryngeal vestibule or supraglottis. The pharyngotracheal duct (a potential space created along the dorsal aspect of the arytenoid swellings) was described as the lumen communicating the hypopharynx above to the trachea below. Kallius[1] designated the glottic level as the ventral border of the epithelial lamina, dorsal to the laryngeal cecum, and he concluded that the eventual separation of the epithelial lamina would produce the infraglottic cavity above the level of the pharyngeal floor.

In contrast to Kallius,[1] Frazer[2] stated that the tracheal opening (the sagittal slit along the pharyn-

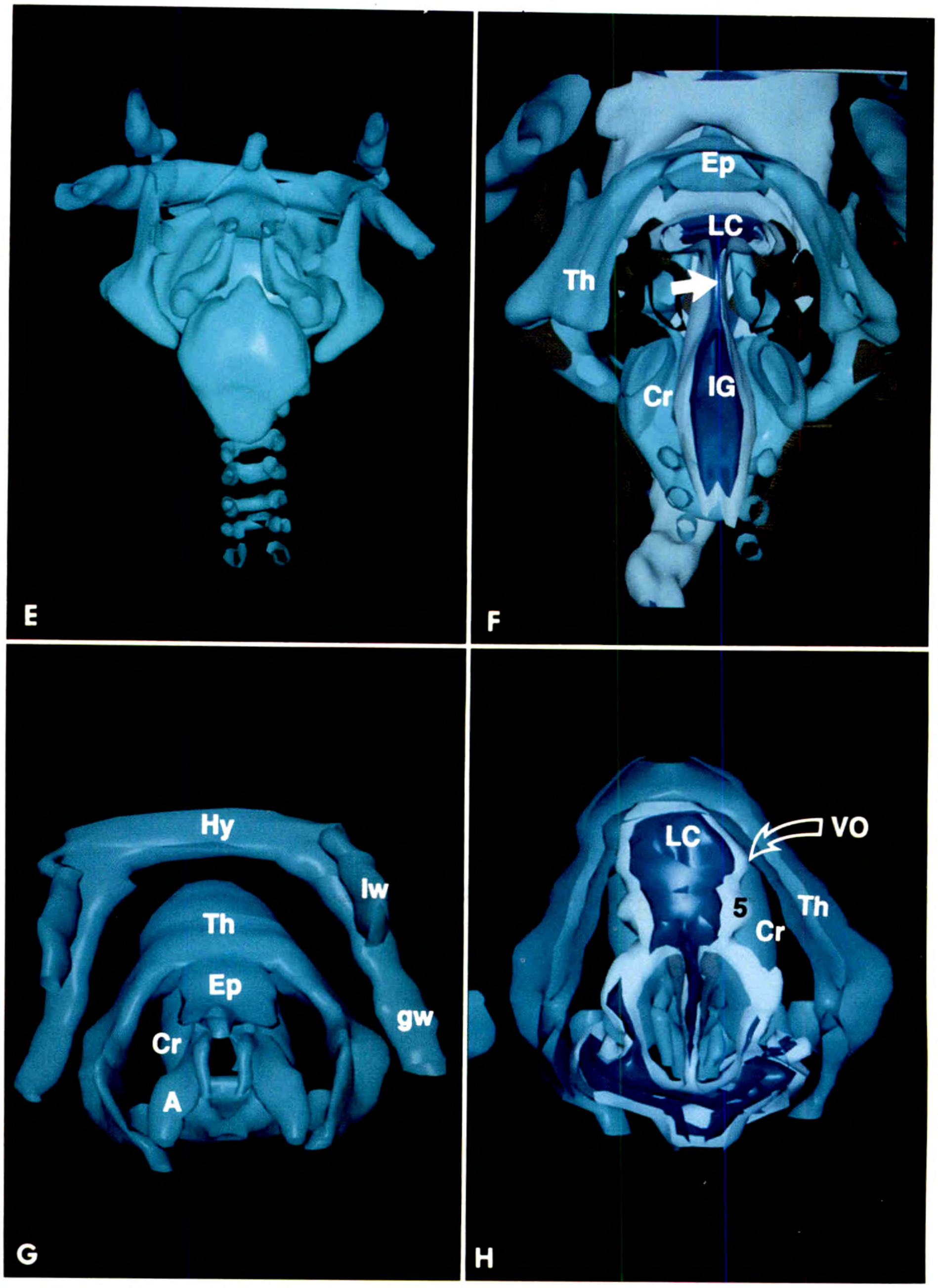

Fig 11, continued. E) 3-D reconstruction of laryngeal cartilages viewed dorsally. Note incomplete dorsal fusion of tracheal rings. Posterior cricoid lamina is fully fused and chondrified. F) Coronal section of E. This section correlates with histologic section seen in B. Early communication between LC and IG is shown by solid white arrow. G) Cephalodorsal view of laryngeal cartilages. Hy — hyoid, Ep — epiglottis, lw — lesser wing of hyoid, gw — greater wing of hyoid. H) Axial section of G at level of laryngeal ventricles. Ventricular outgrowths (VO) are seen as bilateral extensions of LC.

geal floor) was the level of the glottis and that the tracheal lumen immediately caudal was the infraglottis. He believed that the sixth branchial arch mesoderm compressed and obliterated the tracheal lumen immediately below the glottic opening in the pharyngeal floor to create the epithelial lamina. Furthermore, Frazer[2] maintained that the rapid proliferation of the sixth branchial arch mesoderm along its cephalodorsal border (arytenoid swelling region) against the epiglottic swelling would "carry the sagittal line [level of the glottis] in the same direction," thus forming the laryngeal cecum. In accordance with Kallius,[1] Frazer[2] believed that the laryngeal cecum eventually became the supraglottic vestibule. The "displaced sagittal line," or the cephaloventral border of the epithelial lamina, became the glottic level in Frazer's description of laryngeal development. Separation of the epithelial lamina gave rise to the infraglottic cavity, below the level of the original pharyngeal floor.

Walander[3] studied rat embryos and fetuses and postulated that the epithelial lamina formed as a median ventrocaudal outgrowth from the pharyngeal floor, cephalic to the tracheal opening. He observed the laryngeal cecum growing ventrocaudally along the ventral order of the epithelial lamina, and he stated that this gave rise to the laryngeal vestibule. In addition, a pharyngotracheal duct formed along the dorsocaudal aspect of the pharyngeal cavity because of the proliferating epithelial lamina. He believed that the pharyngotracheal duct gave rise to the infraglottic cavity by expanding cranially, and hence placed the glottis at the level of the epithelial lamina, between the expanding vestibule craniad and the infraglottic cavity caudad.

In contrast to the foregoing hypotheses, Zaw-Tun[5] showed that no such tracheoesophageal septum exists, even as a transitory structure during laryngeal development. He demonstrated that the separation between the esophagus and trachea resulted from the ventrocaudal outgrowth of the respiratory primordium as a diverticulum from the caudal end of the laryngeal sulcus of the foregut. Zaw-Tun[5] and Zaw-Tun and Burdi[9] further demonstrated that the respiratory diverticulum bifurcates ventrocaudally into lung buds, which in turn draw out a stalk that gives rise cephalocaudally to the glottic opening, the infraglottis, and the trachea. Zaw-Tun[5] showed that the sagittal slit seen along the pharyngeal floor by his predecessors did not lead to the cephalic end of the trachea, but rather is the entrance to the primitive laryngopharynx, the foregut segment between the fourth pharyngeal pouch and the level of the respiratory diverticulum. Compression by the pharyngeal mesoderm in the region of the primitive laryngopharynx obliterates the foregut lumen ventrodorsally as far craniad as the fourth pharyngeal pouch, forming an epithelial lamina with a narrow pharyngoglottic duct along its dorsal border. The laryngeal cecum actively grows in a caudal fashion along the ventral border of the epithelial lamina to a point just above the glottic level. Eventually, the epithelial lamina separates cephalocaudally, bringing the cecal lumen into continuity with the pharyngoglottic duct to form the laryngeal vestibule.

The basis for Zaw-Tun's theory for laryngeal development is consistent with Negus'[10] original concept that the respiratory apparatus develops as a ventral outpouching from the foregut, the entrance of which becomes the glottis. Negus[10] demonstrated in fish and in mammals that the evolution of the larynx shows the early development of a muscular sphincter that surrounds the glottis at the cephalic region of the respiratory diverticulum. Mammals develop both a sphincter and surrounding laryngeal cartilages.

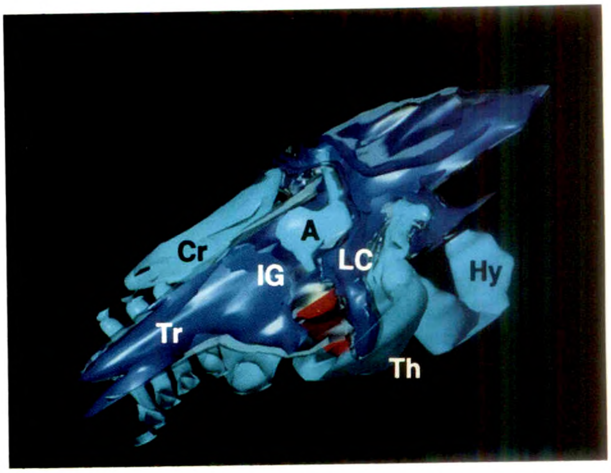

Fig 12. Fetal stage (E18 mouse embryo). 3-D reconstruction that was oriented and sectioned in plane similar to that shown in Fig 11C (midsagittal section). There is full communication between lumen (blue) of infraglottis (IG) and laryngeal cecum (LC). This represents complete recanalization of epithelial lamina. Vocal ligaments are indicated by red.

O'Rahilly and Müller[11] described the developmental stages of the human embryo based on the following sequence of events: in stage 10, the first indication of the respiratory system was represented as a median pharyngeal groove. They described the development of the laryngopulmonary system as a "respiratory tap," with the pulmonary primordium initially appearing as a paired structure. By stage 12, the lung bud grows caudally into the mesenchyme ventral to the foregut. The portion of the mesenchyme that comes to lie between the respiratory and digestive tubes, and that is bounded by the adjacent parts of their epithelium, was believed to constitute the tracheoesophageal septum. They believed that the septum was present from the initial appearance of the lung buds (stages 12 and 13) and was a necessary accompaniment of the normal separation of the respiratory and digestive tubes. Contrary to their predecessors, however, O'Rahilly and Müller claimed that the separation point between the respiratory and digestive systems did not migrate rostrally, but rather remained at a constant somitic-vertebral level, while the tracheal bifurcation descends. Therefore, rather than subscribing to an "ascending tracheoesophageal septum," O'Rahilly and Müller described in essence a "descending tracheoesophageal septum" as the epitheliomesenchymal separation becomes longer by addition at its caudal end. O'Rahilly and Müller described the presence of an epithelial lamina that eventually recanalized to give rise to the laryngeal vestibule. In contrast to Zaw-Tun, however, they described the formation of a "pharyngotracheal duct" and a "vestibulotracheal duct."

Müller et al[12] made interesting observations con-

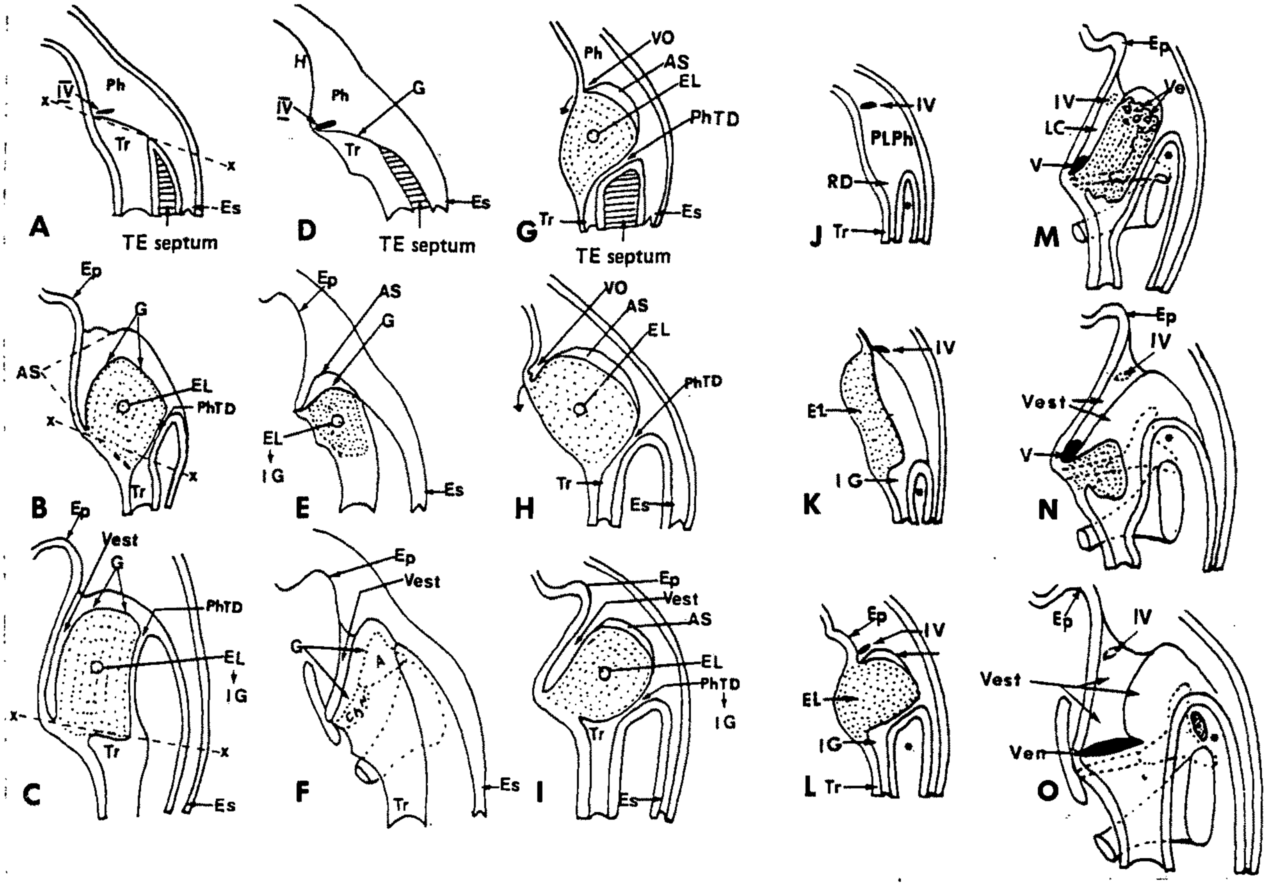

Fig 13. Four historical concepts of development of laryngeal cavity. Kallius[1] (A-C), Frazer[2] (D-F), and Walander[3] (G-I) accepted concept of tracheoesophageal septum originally proposed by His.[4] Zaw-Tun and Burdi[9] (J-O) showed that tracheoesophageal bifurcation was separated from level of fourth pharyngeal pouch by foregut segment called primitive laryngopharynx (PLPh). Stippling indicates epithelial lamina (EL) or area of fusion believed to temporarily obliterate part of laryngeal cavity. x–x plane represents what Kallius[1] assumed to be original level of pharyngeal floor. (Reprinted with permission.[9])

cerning the formation and dissolution of the epithelial lamina. They demonstrated that after fusion of the lateral pharyngeal walls, the lamina increases in surface area and in thickness, with cells arranged irregularly. In stage 23, disruption of the epithelial lamina was shown to be an active process that comprised rearrangement and growth, but not the loss of cells.

DEVELOPMENT OF LARYNGEAL VESTIBULE

Summary of Critical Morphological Events. The respiratory primordium is an epithelial thickening along the ventral aspect of the developing foregut region that appears during stage 11. Anatomically, the respiratory primordium is caudal to the level of the developing fourth pharyngeal pouch (Fig 2).

In stage 12, the foregut lumen expands into the respiratory primordium region to form the respiratory diverticulum (Fig 3A). The cephalic end of the respiratory diverticulum is called the primitive pharyngeal floor. The segment of foregut between the primitive pharyngeal floor and the fourth pharyngeal pouch, or the level of the pharyngeal floor, is called the primitive laryngopharynx. The primitive laryngopharynx will eventually develop into the adult supraglottis. Later in stage 12, bronchopulmonary buds appear as lateral expansions that emerge bilaterally from the respiratory diverticulum (Fig 3B).

The caudal end of the respiratory diverticulum (which will become the carina) is attached to the cephalic aspect of the septum transversum (Fig 3A). We suggest that the respiratory diverticulum is subject to the dynamic forces of the rapidly expanding heart and liver adjacent to the septum transversum. As a result, stages 13 and 14 are characterized by the lengthening of the bronchopulmonary buds from the respiratory diverticulum. The respiratory diverticulum will give rise to the glottis, infraglottis, carina, and the tracheobronchial system, as follows.

1. The rima glottidis develops from the entrance into the respiratory diverticulum or the primitive pharyngeal floor.

2. The infraglottis develops from the cephalic aspect of the respiratory diverticulum. It takes on a

conical shape with the continued descent of the bronchopulmonary buds and carina from the respiratory diverticulum. The infraglottis has a characteristic upright-triangle shape when sectioned in a coronal plane (Fig 6F), and an inverted-triangle shape when sectioned in a midsagittal plane (Fig 6G). Bilateral compression of the caudal aspect of the primitive laryngopharynx, first seen in Fig 5C, contributes, in part, to the characteristic shape of the infraglottic lumen.

3. The carina develops from the caudal aspect of the respiratory diverticulum, and is anatomically located at the bifurcation of the main stem bronchi (Figs 4B and 5B).

4. The bronchopulmonary buds differentiate into the lung primordium. Dorsocaudal descent of the bronchopulmonary buds pulls the level of the carina inferiorly and gives rise to the main stem bronchi (Fig 4A). Further differentiation of the bronchopulmonary buds gives rise to the superior, middle, and inferior lobar bronchi on the right side (Fig 6E).

5. The trachea arises from the continued caudal descent of the carina from the cephalic end of the respiratory diverticulum, or infraglottis. Anatomically, the lumen is uniformly circular. Phylogenetically, it appears subsequent to the presence of the infraglottis and the lung primordium.

The primitive laryngopharynx, which was seen as early as stage 12, becomes compressed bilaterally because of the rapidly enlarging laryngeal mesodermal anlagen, and the lateral walls are brought together in a ventrodorsal direction. The fusion of the lateral walls of the primitive laryngopharynx gives rise to the epithelial lamina seen in stages 14 and 15. Figures 6F and 7B demonstrate the early formation of the epithelial lamina as the ridge seen along the ventral aspect of the primitive laryngopharynx. In addition to the enlarging mesodermal anlagen of the developing larynx, Zaw-Tun and Burdi[9] have suggested that the enlarging fourth branchial arch artery compresses the ventral aspect of the pharyngeal floor of the foregut and gives rise to the arytenoid swellings (Fig 6F, open black arrows), and the enlarging third branchial arch artery participates in giving rise to the hypobranchial eminence (not seen in diagram).

During stages 16 to 18, the epithelial lamina completely obliterates the lumen of the primitive laryngopharynx, except for the narrow pharyngoglottic duct along its dorsal aspect. The pharyngoglottic duct bridges the hypopharynx with the infraglottic region (Fig 8I). Also seen in stage 16 is the laryngeal cecum, a triangle-shape lumen, which is bounded by the arytenoid swellings dorsally and by the epiglottic swelling (which is derived from the dorsal aspect of the hypobranchial eminence) ventrally (Fig 8I,K). The laryngeal cecum descends caudally along the ventral aspect of the epithelial lamina until it reaches the level of the glottis by stage 17 (Fig 9A,C).

In stages 19 through 23, recanalization of the epithelial lamina begins along its cephalodorsal region, and advances in a ventrocaudal direction (Fig 10A). This brings the pharyngoglottic duct into communication with the laryngeal cecum, thus forming the laryngeal vestibule, or supraglottis. The last region to recanalize is the ventral aspect of the glottic level (Fig 11C). Lateral expansions originating from the distal aspect of the laryngeal cecum occur bilaterally to form the vestibular outgrowths (Fig 11H). These will form the adult laryngeal ventricles.

Correlation With Developmental Events in Human Larynx. There is no evidence in the reconstructions presented in this paper that the separation between the trachea and esophagus results from an ascending septal process as proposed by His.[4] The foregut length between the tracheoesophageal separation (this is analogous to the level of the primitive pharyngeal floor; Fig 3A) and the level of the fourth pharyngeal pouch is equivalent to the primitive laryngopharynx. If one measures the length of the primitive laryngopharynx over time, ie, at 22 somites (Fig 3B), 28 somites (Fig 4A), 35 somites (Fig 5A), and 36 somites (Fig 6F), this distance does not decrease (as suggested by His[4]), but, to the contrary, increases. His[4] claimed that the trachea resulted from an "ascending tracheoesophageal septum" from the respiratory diverticulum to the floor of the pharynx (or fourth pharyngeal pouch). In contrast, this study shows that the supraglottis forms in the region of the primitive laryngopharynx, and the trachea results from the continued caudal descent of the bronchopulmonary buds and carina from the cephalic end of the respiratory diverticulum or infraglottis.

On the basis of the findings of this paper, the embryogenesis of the mouse larynx most closely resembles the theories advanced by Zaw-Tun[5] and Zaw-Tun and Burdi,[9] who also sought to discredit the concept of an "ascending tracheoesophageal septum." Therefore, critical events seen in murine laryngeal development were described according to the staging system of human development advanced by Zaw-Tun[5] and Zaw-Tun and Burdi.[9]

The interpretations and conclusions made in this study are likely more accurate than the descriptions made by the classic authors (as cited in Review of Literature) because 1) computer-assisted 3-D reconstructions provide a precise quantitation of morphological events — a technology not used in the traditional developmental anatomic studies, and 2) human specimens available in the Carnegie Collection for previous studies were retrieved from a heterogeneous population of fetal abortuses, whereas the embryonic material used in this study was derived from a homogeneous population of CBA/C57 hybrid mouse embryos. In addition, harvested mouse embryos (obtained by cesarean sectioning) were cut in several standard planes of section (axial, coronal, and sagittal), allowing a more precise localization of specific structures and a comparison among embryos of a specific age. Comparing a solid model reconstruction of a given age — for example, sectioned in the midsagittal plane — with a histologic midsagittal section of a similar-stage embryo allowed us to determine the accuracy and reliability of using the computer image to study anatomic changes over both space and time.

Given this multidimensional approach, the empiric descriptions have been based on more extensive observations of a greater range of material than those of our predecessors. The analysis of these data confirms Zaw-Tun's theory of human laryngeal development and rejects the concept of an ascending tracheoesophageal septum. The present study, however, is subject to the same limitations encountered by the classic authors; ie, without the support of specific controlled mesenchymal-epithelial studies of the developing larynx, one can only speculate that the morphological changes seen are due to an increased epithelial and/or mesenchymal cell proliferation in the regions previously described.

Correlation With Human Congenital Malformations. Congenital anomalies result from dysmorphogenesis, producing abnormal anatomic structures that display characteristics seen at an earlier stage of development. As discussed previously, the trachea appears phylogenetically later in development than either the infraglottis or the bronchopulmonary buds. This observation can help to explain why congenital tracheal agenesis, atresia, or stenosis in the newborn is usually associated with an anatomically normal subglottic region and lung parenchyma. In addition, the blood supply to the developing trachea (ie, the innominate and bronchial arteries) can be compromised as the foregut is rapidly stretched by the morphological forces previously mentioned (Zaw-Tun, personal communication, 1991).

The infraglottis remains patent throughout embryonic and fetal development (Figs 6G, 8I, 9A, 10A, 11A, and 12). The constant characteristic shape of the infraglottis has made it a useful landmark to evaluate the temporal-spatial relationships of adjacent structures. In addition, the term "congenital subglottic stenosis" is a diagnosis often considered clinically in the neonate with stridor; however, on the basis of this anatomic study, the infraglottic region does not appear to undergo the obliterative process that is normally seen in the development of the supraglottic region (ie, the epithelial lamina). This finding suggests a possible cause of congenital supraglottic or glottic stenosis; however, the cause of an isolated congenital subglottis stenosis is not readily apparent from the critical stages identified in normal murine laryngeal development.

It is important to note, however, that the infraglottic lumen does continually dilate and expand during normal laryngeal development. This can be seen by comparing the infraglottic region viewed by sagittal sectioning in Figs 10A and 11A. Therefore, even though the infraglottic lumen always appears to be patent during normal laryngeal development, developmental arrests at an earlier stage (which would have associated supraglottic or glottic anomalies) may not allow for the normal continued dilation and expansion of the infraglottic region. This clinically may appear as congenital subglottic stenosis, but it would not be an isolated finding.

Zaw-Tun[13] used the classification of Smith and Bain[14] to describe the cause of congenital laryngeal atresias seen in human development. Smith and Bain's description of type 1 atresia involved the complete closure of the larynx above the glottic level (eg, no supraglottic vestibule). They described a narrowed infraglottic lumen, with a complete supraglottic stenosis except for the minute pharyngoglottic duct along the dorsal aspect of the epithelial lamina. A developmental arrest in the separation of the epithelial lamina prior to its recanalization seen in stage 19 can give rise to a type 1 atresia (Fig 14[13]). Type 2 atresias were described as a partly formed vestibule with identifiable sinuses (ie, laryngeal cecum, pharyngoglottic duct), which appear above an incompletely separated portion of the epithelial lamina (Fig14). Chronologically, type 2 atresias may occur between stages 19 and 23, during the active recanalization of the epithelial lamina. Type 3 atresias ("glottic type") present the epithelial lamina remnant as a perforated membrane of variable thickness at the level of the vocal cords. It obstructs the glottic opening that separates the almost fully formed vestibular cavity and its sinuses from the well-formed infraglottic lumen (Fig 14).

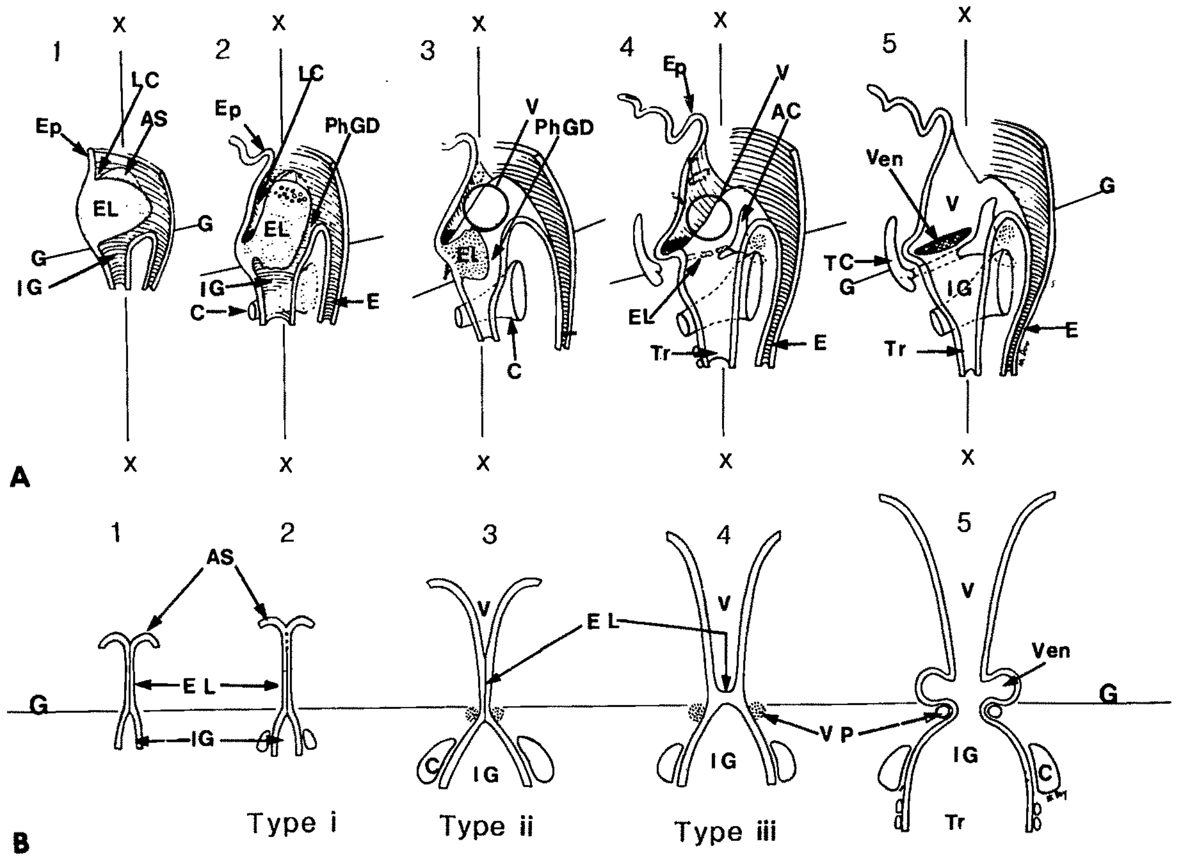

Fig 14. Developmental stages of human laryngeal cavity. A) Modified from reconstructions cut in midsagittal plane. Specimens from Zaw-Tun[13]: 1 — specimen SH77, 35 days, 2 — specimen SH81, 50 days, 3 — specimen EH479, 60 days, 4 — specimen EH277, 70 days, 5 — specimen EH1545, 84 days, Ep — epiglottis, LC — laryngeal cecum, PhGD — pharyngoglottic duct, E — esophagus, AC — arytenoid cartilage, TC — thyroid cartilage. B) Diagrams of corresponding coronal sections along plane x–x, showing separation of epithelial lamina (EL) and formation of vestibule. Arrest of EL separation results in supraglottic atresia of type 1, 2, or 3. AS — arytenoid swelling, G — glottic level, IG — infraglottis, C — cricoid, V — vestibule, Tr — trachea, Ven — ventricle, VP — vocal process. (Reprinted with permission.[13])

DEVELOPMENT OF LARYNGEAL SKELETON AND MUSCULATURE

Summary of Critical Morphological Events. The laryngeal mesodermal anlagen appear as a wedge-shape area flanking the primitive laryngopharynx during stage 14. Further differentiation of the laryngeal mesodermal anlagen gives rise to two morphologically distinct regions in stage 16: a hyoid region and a cricothyroid region. The thyroid anlage appears to have a U-shape configuration, which gives rise inferiorly to three distinct regions of the cricoid anlage: two dorsolateral and one ventromedian (Fig 8M).

Chondrification centers of the laryngeal mesodermal anlagen first appear along the ventral aspect of the hyoid anlage (Fig 9D,F,G) and along the ventral aspect of the cricoid anlage in stages 17 and 18. In addition, lateral and dorsal fusion of the cricoid anlage occurs in the same stages (Fig 9E,H). Complete chondrification of the laryngeal cartilages occurs by stages 19 to 23.

Differentiation of the intrinsic laryngeal muscles is apparent by stage 19 (E15 mouse embryo; Fig 10C).

Correlation With Developmental Events in Human Larynx. The observations made in this embryologic series with regard to the appearance of the laryngeal cartilages and musculature are consistent with those of other investigators of human laryngeal development. The main area of controversy concerns the development of the cricoid cartilage. Fleischmann in 1820 and Arnold in 1851 (cited by Zaw-Tun and Burdi[9]) reported that the cricoid developed from chondrifying halves that initially fused dorsally and then fused ventrally. Kallius[1] also described bilateral chondrification centers that initially met ventrally, and then met dorsally. Soulie and Bardier[15] reported in 1907 that the bilateral chondrification regions met simultaneously at both their dorsal and ventral aspects. Zaw-Tun and Burdi[9] believed that chondrification started in the ventral arch of the cricoid, and extended dorsally bilaterally on each side of the infraglottis to "meet behind the lumen." This present study confirms a single ventral chondrification center of the cricoid anlage in the E14 mouse embryo; however, by E15, complete chondrification of all laryngeal cartilages (except for the epiglottis) has occurred. Further work is necessary to elucidate the precise sequence of chondrification in the mouse embryo.

Correlation With Human Congenital Malformations. Congenital laryngeal and laryngotracheoesophageal clefts have been classified by various authors.[16,17] They are communications between the laryngopharynx and the larynx. At least three types are seen: type 1, failure of interarytenoid and aryepiglottic muscles to develop, which will result in an interarytenoid cleft and flaccid aryepiglottic folds; type 2, incomplete fusion of the cricoid dorsally, leaving a central foramen in the dorsal lamina, or a median notch along its caudal border, or a configuration of both conditions; and type 3, failure of the lateral rami of the cricoid to meet dorsally, which produces a complete laryngeal cleft associated with completely separate arytenoids. All three of these malformations can result from either an incomplete fusion of the cricoid anlage dorsally, or an incomplete chondrification of the dorsal cricoid region.

SUMMARY

This paper has demonstrated that all the critical stages identified in human laryngeal development are also present during murine embryogenesis. In addition, the series of embryologic events identified during murine laryngeal development have helped to resolve the controversy of His'[4] misconception of an "ascending tracheoesophageal septum." Human anomalies were correlated to critical stages in the development of the mouse larynx. The possible role that arrests of these critical stages may play in laryngeal anomalies was discussed. Future research with this murine model for the development of the larynx may help elucidate the role that growth factors play during laryngeal development. Furthermore, in vitro culture and manipulation of murine laryngeal anlagen may allow the actual testing of the role that arrested development plays in the causation of malformations.

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PROLONGED INTUBATION INJURIES OF THE LARYNX: ENDOSCOPIC DIAGNOSIS, CLASSIFICATION, AND TREATMENT

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LARYNGOLOGY

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PROLONGED INTUBATION INJURIES OF THE LARYNX: ENDOSCOPIC DIAGNOSIS, CLASSIFICATION, AND TREATMENT

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Laryngeal trauma from prolonged endotracheal intubation occurs in patients of all ages. Most changes are superficial and heal quickly. Injuries that are found consistently during intubation include nonspecific changes, edema, granulation tissue, ulceration, and other miscellaneous injuries. In this paper significant, severe, and lasting trauma of the larynx has been classified on the basis of the known factors in pathogenesis, observations made at endoscopy, and photographic documentation. This classification has required introduction of new descriptive terminology: "tongues of granulation tissue," "ulcerated troughs," "healed furrows," and "healed fibrous nodule." During intubation the degree of injury can be precisely assessed under general anesthesia by using telescopes for image magnification, thus assisting a decision whether to continue intubation or perform tracheotomy to minimize long-term morbidity. Changes that are found after extubation result from granulation tissue, ulceration, or a combination of both and have been illustrated on flow charts; a knowledge and understanding of these sequelae allows them to be identified by both indirect and direct laryngoscopy so that treatment can be planned.

KEY WORDS — endoscopy, intubation, laryngeal trauma, larynx.

Endotracheal intubation has replaced tracheotomy as primary airway control for prolonged ventilation in patients of all ages, yet both acute and chronic changes in the larynx caused by the tube continue to cause morbidity.[1-6] This paper examines the pathogenesis, emphasizes the endoscopic recognition, offers a classification, and reviews the principles of treatment of these laryngeal injuries. Four changes not previously highlighted are depicted, and new descriptive terms are introduced for them. The observations are based on experience gained over many years from approximately 400 endoscopic procedures in infants and children and 300 in adults performed each year, many of the patients having changes caused by prolonged intubation. Documentary evidence was analyzed from 35 mm still photography and from videorecording at endoscopy. Injuries to the trachea caused by pressure from the cuff of the tube are now seldom seen with the use of high-volume, low-pressure cuffs, but there has been no corresponding decrease in the incidence of laryngeal damage.[2,3,5] Because airway obstruction due to laryngeal or subglottic stenosis presents to the laryngologist many weeks or months after the period of prolonged intubation, intensive care physicians may remain unaware of the incidence of late complications.

Changes Due to Tube Pressure. The important consideration in mucosal injury is capillary perfusion. When pressure from the firm walls of the endotracheal tube is greater than mucosal capillary pressure, ischemia occurs, first with irritation, then congestion, edema, and eventually ulceration.[4,5] Progressive ulceration[6] causes perichondritis, chondritis, and finally necrosis involving the cricoarytenoid joints and the cricoid cartilage.[7] This stage of ulceration at the site of pressure necrosis from an indwelling anesthetic tube can be likened to a "laryngeal bedsore."

Granulation tissue proliferates at the margins of the injured area and may persist even after removal of the tube as ulcerative lesions heal by secondary intention. A localized intubation granuloma is due to persistent granulation tissue.[8,9] Extensive or deep changes promote formation of new collagen that matures to fibrous, firm scar tissue; thus, chronic intubation changes, including subglottic stenosis and posterior glottic stenosis, cause progressive narrowing of the airway as the scar slowly contracts.

An endotracheal tube will always lie in and exert pressure on the posterior larynx,[3,5] affecting three possible sites of major damage: first on the medial surfaces of the arytenoid cartilages, cricoarytenoid joints, and vocal processes, second in the posterior glottis and interarytenoid region, and third in the subglottis involving the inner surface of the cricoid cartilage, usually the posterior lamina. The circumference of the subglottic space is especially vulner-

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able in infants and small children because of its relatively small caliber.

Factors in Intubation Trauma. Many factors have been identified as contributing to laryngeal intubation trauma.

1. An abnormal larynx is more prone to damage than a normal larynx, for example, a larynx crushed by external trauma or a subglottic larynx with acute inflammatory swelling caused by acute laryngotracheitis ("croup").[10]

2. Another factor is trauma sustained during intubation performed in an emergency, by an unskilled person, following repeated intubations, associated with improper use of an introducer, or when unusual anatomy causes intubation difficulty. Tube movement causes trauma from transmitted ventilator movement, during suctioning or from coughing or swallowing, and aggravates any existing injury.

3. Impairment of the mucociliary clearing mechanism can be due to excessive secretions, stasis of secretions, or bacterial contamination.[1,11] Other factors include the presence of the tube itself, trauma to the tracheobronchial mucosa from suctioning, and the effect of drugs.

4. Gastroesophageal reflux with spillover and aspiration of acid into the larynx and subglottic region aggravates the local injury and predisposes to infection, ulceration, and granulation tissue.[8] Reflux of acid gastric contents is made more likely by the presence of a nasogastric tube that not only irritates the lower esophagus but can cause pressure necrosis and ulceration in the postcricoid region.[12]

5. Bacterial infection occurs quickly in all tracheotomy wounds, especially in the immunodeficient patient or those in poor general health. When a tracheotomy is performed below a larynx already the site of intubation trauma, subsequent contamination of the tracheotomy stoma can prolong laryngeal healing and influence scar formation. Care should be taken to minimize infection in these circumstances.[13]

6. Acute or chronic diseased states[4] with altered levels of consciousness, poor tissue perfusion, and hypoxia are associated with more severe changes, as are anemia, hypotension, hypoxemia, and heart, kidney, or liver failure.

The two most important factors contributing to the severity of intubation damage are the duration of intubation and the physical characteristics of the tube, namely size and unfavorable shape.[1,5]

7. There is no universal agreement about the "safe" or desirable duration of intubation. In most adults a period of 5 to 7 days can be considered a reasonable time before a decision should be made as to whether to continue intubation or change to a tracheotomy. This depends, to some extent, on the patient's general condition and whether removal of the tube might soon be possible. Endoscopic assessment of the nature and degree of intubation trauma will assist in this decision. In infants the time for prolonged intubation before there is risk of irreversible damage is longer than in adults. With skilled care in neonatal intensive care units (ICUs) there is almost no limit to the time of intubation, which can extend for many weeks and be associated with only a low incidence of problems. The laryngeal cartilages in infants may yield and mold to pressure more than in older individuals.[14]

8. The external diameter, shape, firmness, and composition of the endotracheal tube are vital in the development of laryngeal trauma. There is greater pressure on the surrounding posterior laryngeal structures with a tube of excessive diameter.[1,5] It seems reasonable that the upper limit should be 8.0 mm inside diameter in males and 7.0 mm in females, but in practice the tube size must be chosen for each individual patient. Infants and children up to 7 or 8 years of age should have an uncuffed tube with a diameter that under ideal circumstances allows an air leak in the subglottic space with approximately 20 cm of water ventilation pressure.[15]

Specially shaped tubes[5,11] have been recommended to minimize undue pressure exerted by the curvature of conventional tubes in the posterior and lateral larynx, but in practice, although these thoughtfully designed tubes are available, they are seldom used.

Plastic tubes made, for instance, of siliconized rubber are smooth-walled and less irritating; on the other hand, their softness predisposes thin-walled tubes for pediatric use to be more easily compressed. Integration of a thin metal wire spiral within the wall of the tube improves the ability to withstand pressure, but the manufacturing process is expensive.

Injuries of the tracheal wall from the inflated cuff of the endotracheal tube depend on the position and pressure of the cuff, but are now seldom seen,[2] since pressure in the balloon is minimized by the use of a high-volume, low-pressure cuff.

Identification of the above predisposing factors, together with the findings at endoscopic examination, is important in the overall assessment of laryngeal trauma in each patient.

ENDOSCOPIC ASSESSMENT

The knowledge that prolonged laryngeal intuba-

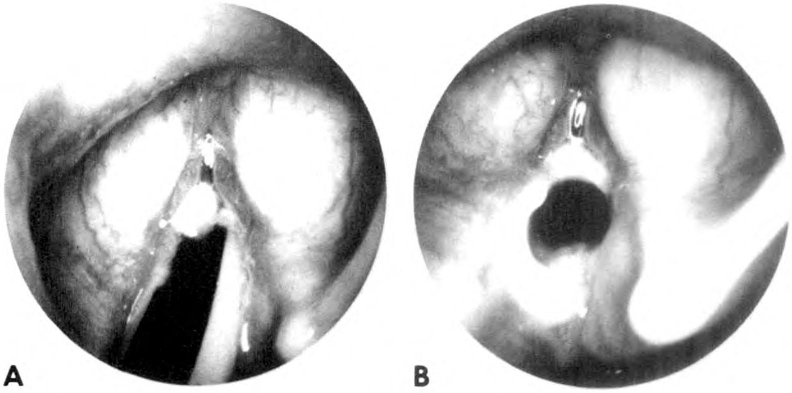

Fig 1. Contrast of view with tube in situ and after removal. A) With endotracheal tube in position, edematous protrusion of ventricular mucosa can be seen on each side. There appear to be thick secretions on tube. B) With tube removed, extent of intubation injury can be appreciated more fully.

tion will cause significant morbidity even in an ICU with the best facilities and personnel and with endotracheal tubes of relatively small diameter that exert minimum pressure (yet large enough to ensure adequate ventilation) poses the question of what can be done to minimize the acute injuries and their subsequent chronic sequelae. Laryngeal damage begins during the first few hours and continues with each further day of intubation.[4,6]

Experience with adults, children, and infants has shown that the nature and degree of laryngeal trauma during prolonged intubation can be precisely assessed by using rigid telescopes for image magnification with the patient under general anesthesia[16] so that the endotracheal tube can be temporarily removed to allow complete examination of the upper airway. Examination in the ICU with the endotracheal tube in situ, without general anesthesia, and with a flexible fiberscope cannot be recommended; the presence of the tube obscures the vital areas (Figs 1 and 2). Both the posterior glottis and the subglottis must be assessed after removal of the tube by means of the superb clarity provided only by rigid telescopes.

Endoscopic Assessment During Intubation. There are diverse opinions regarding the duration of intubation before tracheotomy. Although it is neither possible nor advisable to formulate "rules" for the duration of intubation, in general, experience indicates a need for endoscopic assessment in most adults after 5 to 7 days, in children after 1 to 2 weeks, and in infants only when attempted extubation has been unsuccessful.

Intubation can be relatively safely continued, possibly with an endotracheal tube of smaller diameter, when the changes seen include edema in the vocal folds, edematous protrusion of the ventricles, surface mucosal ulceration, generalized erythema, and minor granulation tissue at the vocal process; of particular importance is the absence of deep ulceration indicating perichondritis. If the tube can be removed at this stage, the changes can be expected to resolve without any treatment. However, changes that indicate severity and the need to consider tracheotomy include deep ulceration through the perichondrium into the cartilage of the arytenoid and the cricoid and into the cricoarytenoid joint, and widespread concentric ulceration in the subglottic region. In other cases, when removal of the tube is followed by obstruction caused by large masses of granulation tissue or worsening edema, reintubation, possibly followed by tracheotomy, becomes an urgent necessity.

The information obtained by thorough endoscopy assists in an informed and rational decision whether to attempt extubation, continue intubation for a further period, possibly with a tube of smaller diameter, or perform tracheotomy. In addition, endoscopy allows the option of removal of obstructing granulation tissue (which may improve the airway prior to attempted extubation) and facilitates culture of aspirated secretions or pieces of granulation tissue.

Endoscopic Assessment After Extubation. The clinical features of intubation changes become manifest at different times after removal of the tube, depending on the nature of the trauma.

1. Large "tongues" of granulation tissue produce a valvelike obstruction (Fig 2) almost immediately after extubation. Smaller amounts of granulation tissue are unlikely to contribute to airway obstruction.
2. Reactive glottic or subglottic edema causes increasing obstruction in the first minutes or hours.
3. Persistent edema and granulation tissue that is not resolving cause partial obstruction and a husky, weak voice in the first few days.
4. An intubation granuloma presents as a husky voice many weeks later.
5. Posterior glottic stenosis or subglottic stenosis due to contracting scar tissue causes increasing obstruction weeks or months after extubation.

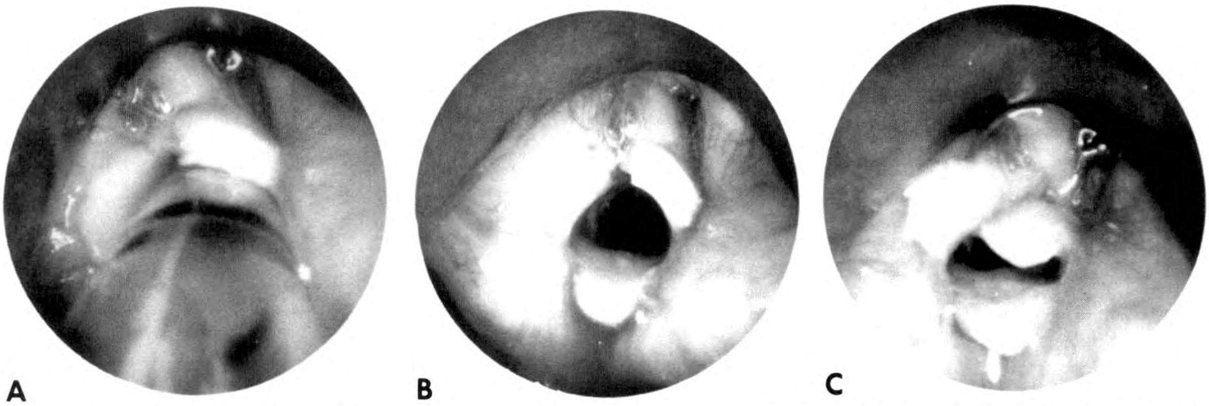

Fig 2. Good view of glottis possible only after removal of tube. A) Endotracheal tube obscures assessment of glottis. B) Immediately after removal of tube, pale, proliferative granulation tissue is visible. C) Some moments later, flaps of granulation tissue are seen to cause partial airway obstruction during respiration.

Some chronic changes can be identified at indirect laryngoscopy using a laryngeal mirror, a 70° or 90° angled rigid telescope, or a flexible fiberoptic laryngoscope. Indirect examination is most important to assess vocal cord movement, but it may be impossible to differentiate a paralyzed vocal cord from a fixed cricoarytenoid joint until mobility of the joint is tested at direct laryngoscopy. Mobility of the arytenoid on the articular facet of the cricoid is seen and "felt" by exerting gentle pressure with a sucker on the medial aspect of the arytenoid cartilage to assess lateral movement, and similarly on the lateral aspect to assess medial movement; in this way fixed or partly fixed joints can be reliably detected. These joint abnormalities may be difficult to demonstrate, but can be suspected from surrounding changes such as posterior glottic stenosis, a healed furrow, or a healed fibrous nodule. The joints can be tested only at direct laryngoscopy.

Therefore, assessment of chronic problems causing persistent voice change or partial airway obstruction after extubation often requires direct endoscopy under general anesthesia with telescopes for special attention to the subglottic and the posterior glottic areas, the medial surfaces and vocal processes of the arytenoids, and the mobility of the cricoarytenoid joints. Serious long-term damage includes posterior glottic stenosis, subglottic stenosis, and decreased mobility or fixation of the cricoarytenoid joints. Many lesser changes can occur and will be described. In patients with obstruction, the airway diameter should be measured in millimeters, and vocal cord movements should be patiently observed during the final stage of laryngoscopy, when anesthesia has been discontinued and muscular tone returns.

GENERAL DESCRIPTION OF INTUBATION CHANGES

The degree of damage differs from patient to patient; some individuals incur minimal injury, whereas others, intubated for the same time, have severe changes. The observed changes can be categorized into five groups: early nonspecific changes, edema, ulceration, granulation tissue, and miscellaneous injuries. Distinctive clinical entities are highlighted in the text in italics, eg, *protrusion of the ventricle*, below.

Early Nonspecific Changes. Hyperemia and edema with inflammatory swelling are the first changes to be seen. Continued intubation promotes patchy areas of superficial ulceration, and granulation tissue begins to form at the edges of the site of pressure and irritation from the tube (Fig 3A).

Edema. Vocal fold edema usually resolves after extubation, but occasionally persists as chronic Reinke's edema with consequent voice dysfunction. Some premature infants who have undergone prolonged intubation may have a weak, husky cry and later a troublesome, permanently husky voice; close examination confirms intractable edematous swelling of the vocal cords (Fig 4I).

Diffuse swelling of the mucosa of the laryngeal ventricles is a common and prominent change during intubation (Figs 1 and 4A) and is best described as *protrusion of edematous mucosa of the ventricle*. This swelling subsides quickly once the tube is removed.

Swelling in the submucosal tissues within the cricoid cartilage can occur slowly and relentlessly after removal of an endotracheal tube, especially in infants and children, and in the postextubation period *subglottic edema* can be the cause of worsening obstruction that may require reintubation and consideration for tracheotomy.

Granulation Tissue. Granulation tissue represents attempted healing and forms at the site of irritation and ulceration caused by tube pressure. *Tongues of granulation tissue* (Fig 3) begin to arise within 48

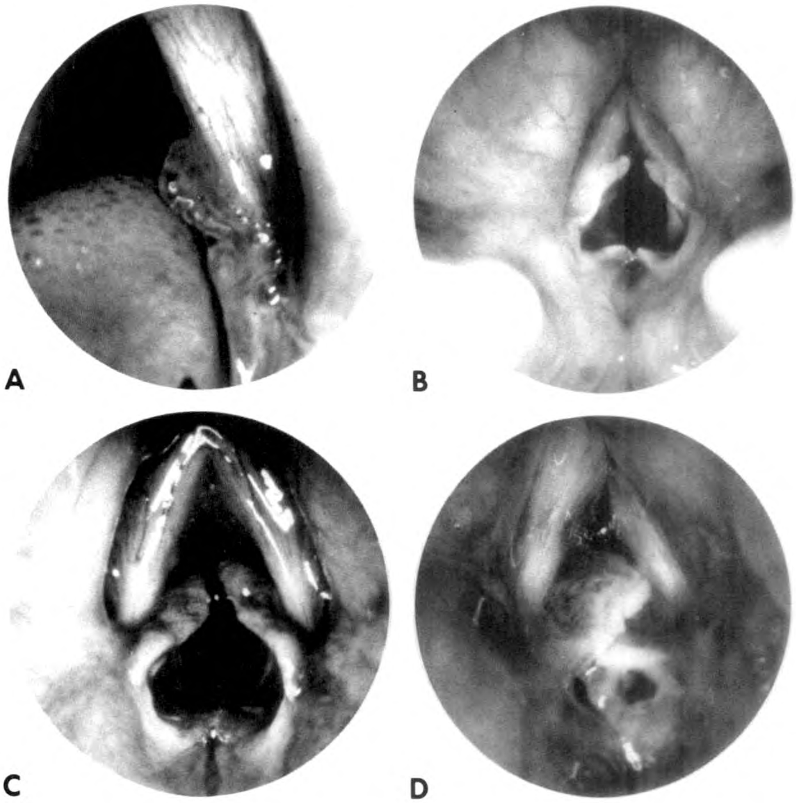

Fig 3. Stages in formation of "flaps of granulation tissue." A) Close-up of granulation tissue beginning to form at right vocal process in response to irritation from endotracheal tube. B) Immediately after removal of endotracheal tube, areas of granulation tissue and pressure ulceration can be seen. C) Typical "tongues of granulation tissue" that encircle endotracheal tube. D) Granulation tissue has proliferated around surface of tube, forming flaplike obstructions. Tube has been removed.

hours from the vocal processes and continue to proliferate, almost encircling the anterior surface of the tube on each side. They often appear as large flaplike tongues (Fig 3D) that can cause severe obstruction after attempted removal of the tube. However, in most cases once the irritation of the tube is removed there is complete resolution. Occasionally it might be necessary to remove the granulation tissue from one side to improve the airway before extubation, and culture of this tissue might be helpful in determining antibiotic treatment.

A mature, rounded *intubation granuloma* (Fig 5) on one or occasionally both sides will form if the granulation tissue persists. In other cases there is almost complete healing, leaving only a small, inconspicuous, firm, permanent *healed fibrous nodule* (Fig 4B) of scar tissue near the vocal process.

Occasionally the tongues of granulation tissue or the ulcerated surfaces become adherent across the midline at or posterior to the vocal processes and subsequently form an *interarytenoid adhesion* (Fig 6), a firm, fibrous band that limits vocal cord abduction.

Granulation tissue forming on the margins of an area of ulceration commonly occurs in the posterior glottis and the subglottic area within the cricoid cartilage. Proliferative granulation tissue following deep ulceration can fill the posterior larynx (Fig 7A) and may eventually form firm scar tissue, thus creating *posterior glottic stenosis*[17-20] (Fig 7). Similarly, contraction of concentric scar tissue in the subglottic region forms *subglottic stenosis*[21,22] (Fig 8). The presence of a posterior central strip of intact mucosa (Fig 9A) with ulceration and granulation tissue only in the lateral aspects of the posterior glottis is a favorable sign that posterior glottic stenosis is less likely to eventuate.

Ulceration. Superficial ulceration covers with mucosa and heals when the tube is removed. Deep ulceration into the perichondrium or cartilage carries the implication of delayed healing and possible future formation of scar tissue causing stenosis of lesser or greater degree. Thus, deep ulceration in the posterior glottic and subglottic space involving the cricoarytenoid joints is likely to lead to airway and/or voice problems.

An *ulcerated trough* (Fig 9A,B) is an obvious wide, deep area of erosion and rounded ulceration on the medial aspect of the arytenoid and cricoid cartilages, often exposing the cricoarytenoid joints on one or both sides, and involving an area that can be seen only after the endotracheal tube has been removed. Weeks or months after extubation, healing and fibrosis lead to a contracted, linear *healed furrow* (Fig 10), usually on both sides and associated with chronic dysfunction or even *fixation of the cricoarytenoid joint*.

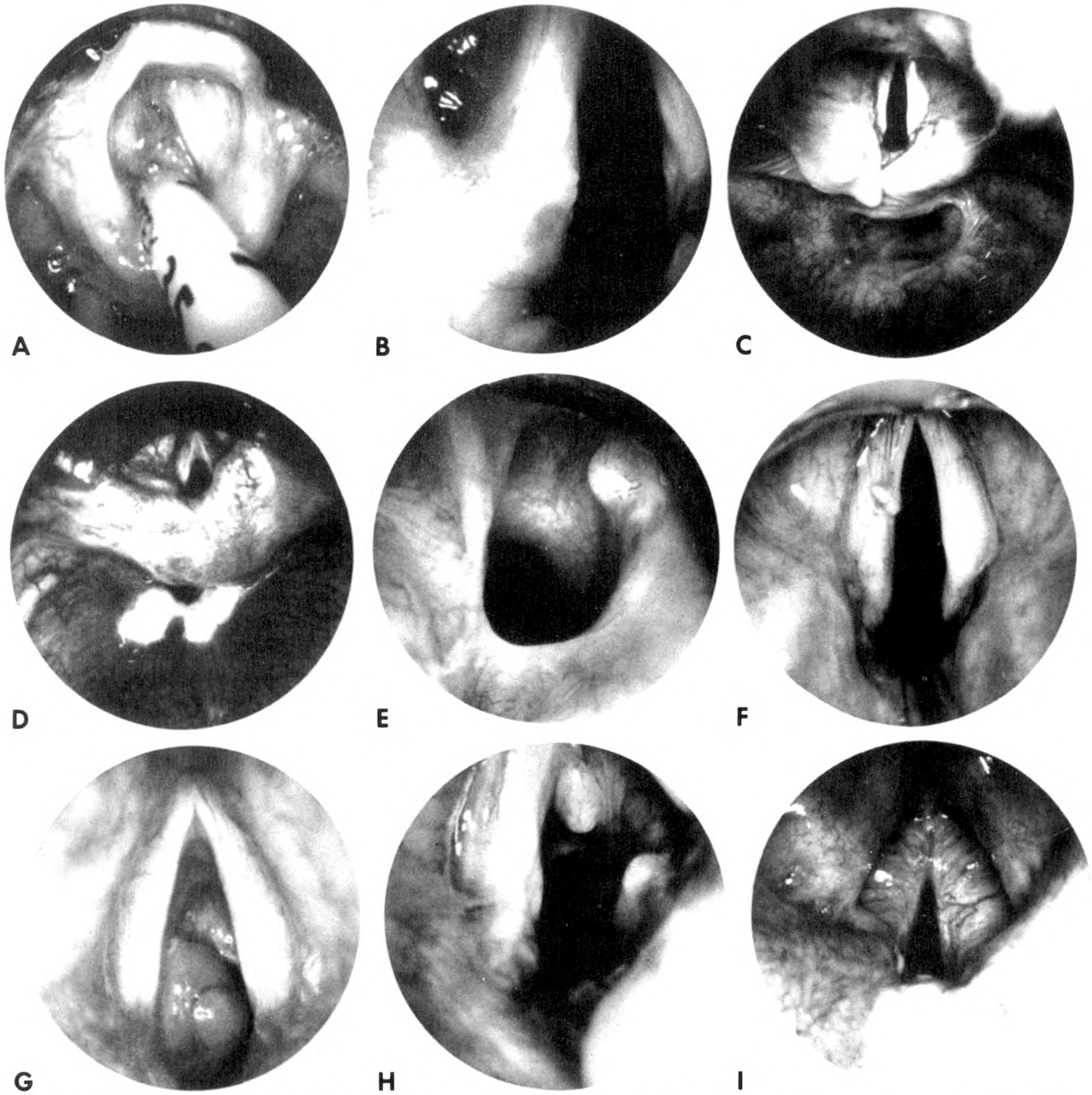

Fig 4. Miscellaneous changes due to intubation, some not classified. A) Protrusion of edematous mucosa from laryngeal ventricles. B) Close-up of healed fibrous nodules just posterior to vocal process of arytenoid on left side. C) Dislocation of left arytenoid cartilage. D) Ulceration in postcricoid region caused by nasogastric tube. E) Subglottic ductal retention cyst and posterior glottic stenosis in infant. F) Irregular scarred central part of left vocal cord, probably healed laceration caused during intubation. G) Large, left subglottic intubation granuloma and anterior acquired subglottic stenosis following prolonged intubation in 10-year-old boy with head injury. H) Large defect of right vocal cord in child who was intubated as neonate. After one difficult intubation it was said vocal cord had been "scraped." I) Chronic edema, congestion, and diffuse scarring of both vocal cords in 2-year-old with weak, hoarse voice. Result of prolonged intubation as premature infant.

In the posterior glottis deep *annular ulceration* (Fig 9C) with or without adjacent granulation tissue is likely to fibrose, scar, and eventually form posterior glottic stenosis, one of the serious long-term sequelae of intubation trauma.

Miscellaneous Injuries. These include dislocation of an arytenoid (Fig 4C),[23] laceration of or bleeding into a vocal cord, damage to intrinsic muscles,[24] and even perforation of the airway with spreading surgical emphysema and infection in the soft tissues of the neck or mediastinum. These injuries may be caused by either the endotracheal tube or the introducer and are more likely after difficult intubation. A nasogastric tube can cause postcricoid ulceration (Fig 4D) — a change that often goes unrecognized.

An acute laceration will usually heal, sometimes with a small permanent scar (Fig 4F); incomplete healing may result in a granuloma in an atypical site (Fig 5B,C). Very rarely, an acute posterior laryngeal pressure injury, especially if associated with ulcer-

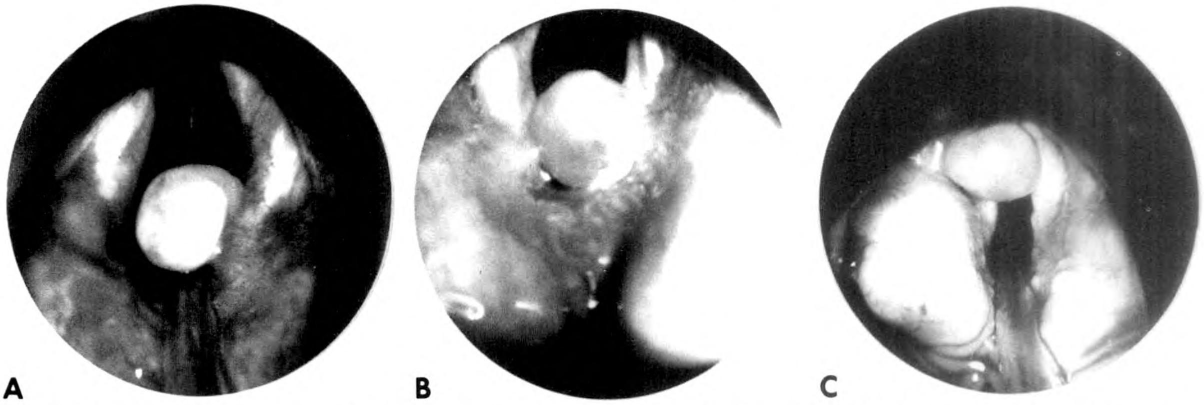

Fig 5. Postintubation granulomas. A) Typical rounded, mature intubation granuloma arising from region of vocal process on right side. B) Similar rounded granuloma, but arising posterior to vocal process on right side. C) Smooth, rounded granulation tissue arising anteriorly on left (atypical site), caused by laceration from endotracheal tube or introducer.

ation in the postcricoid region caused by a nasogastric tube, will cause an infected sinus in the posterior lamina of the cricoid cartilage, or even a fistula draining posteriorly and identifiable at pharyngoesophagoscopy. Other unusual injuries will occasionally be seen (Fig 4H) for which there is no diagnostic category.

SPECIFIC INTUBATION INJURIES

Healing begins as soon as the endotracheal tube is removed. Resolution with regeneration of mucosa, sometimes with squamous metaplasia and some scarring, will return the larynx and the airway to a near-normal state, usually without functional deficit, but persistent infection and granulation tissue leading to scar tissue can eventually cause severe stenosis and life-threatening airway obstruction.

Four new descriptive terms (which have been used above) have been introduced.

Tongues of Granulation Tissue. These occur consistently at the vocal process of the arytenoid cartilages and have already been described above (Fig 3).

Ulcerated Troughs. Ulcerated troughs (Fig 9) are obvious wide, deep areas of acute ulceration eroding into the cartilages in the vicinity of the cricoarytenoid joint at the site of maximum tube diameter. They can be seen only after the endotracheal tube has been removed.

Healed Furrows. Healed furrows (Fig 10) develop from incompletely healed ulcerated troughs, and both are usually bilateral. They are seen in the chronic phase as a scarred linear furrow running craniocaudally on the medial aspect of the arytenoid and cricoarytenoid joint. They are often associated with cricoarytenoid joint abnormalities causing minor but annoying dysphonia. Healed furrows can be difficult to recognize. The appearance at first suggests a defect in the posterior part of the vocal fold (Fig 10B), but close examination with a 30° telescope assists recognition of the typical appearance.

Healed Fibrous Nodule. A healed fibrous nodule is a small, round, persistent area of scar tissue on the edge of the vocal cord near the vocal process. It forms when most of the granulation tissue heals, leaving only a small, often inconspicuous nodule covered by mucous membrane (Fig 4B). Removal of a healed

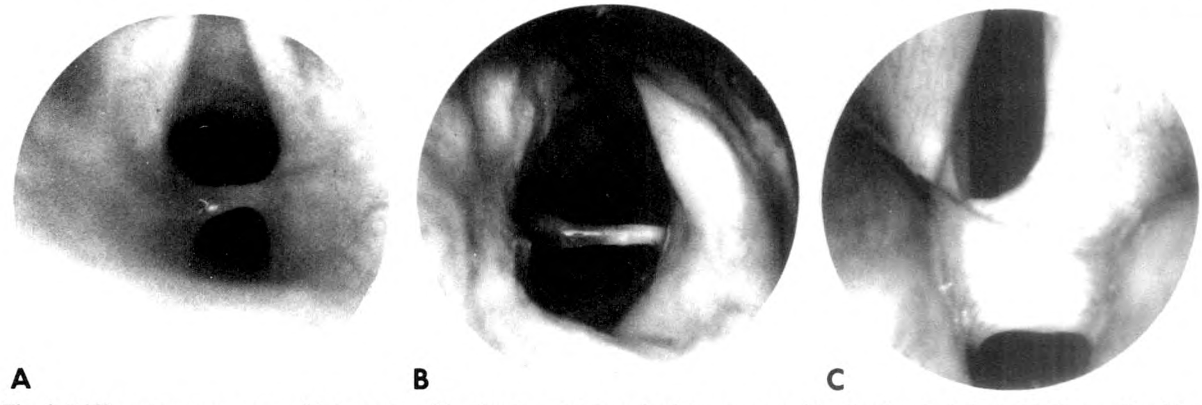

Fig 6. Different appearances of interarytenoid adhesion. A) Acquired interarytenoid adhesion in infant. B) Unusually thin, subglottic interarytenoid adhesion in young woman. C) Mature, thick, long-standing firm interarytenoid adhesion, erroneously diagnosed as bilateral vocal cord paralysis for many years.

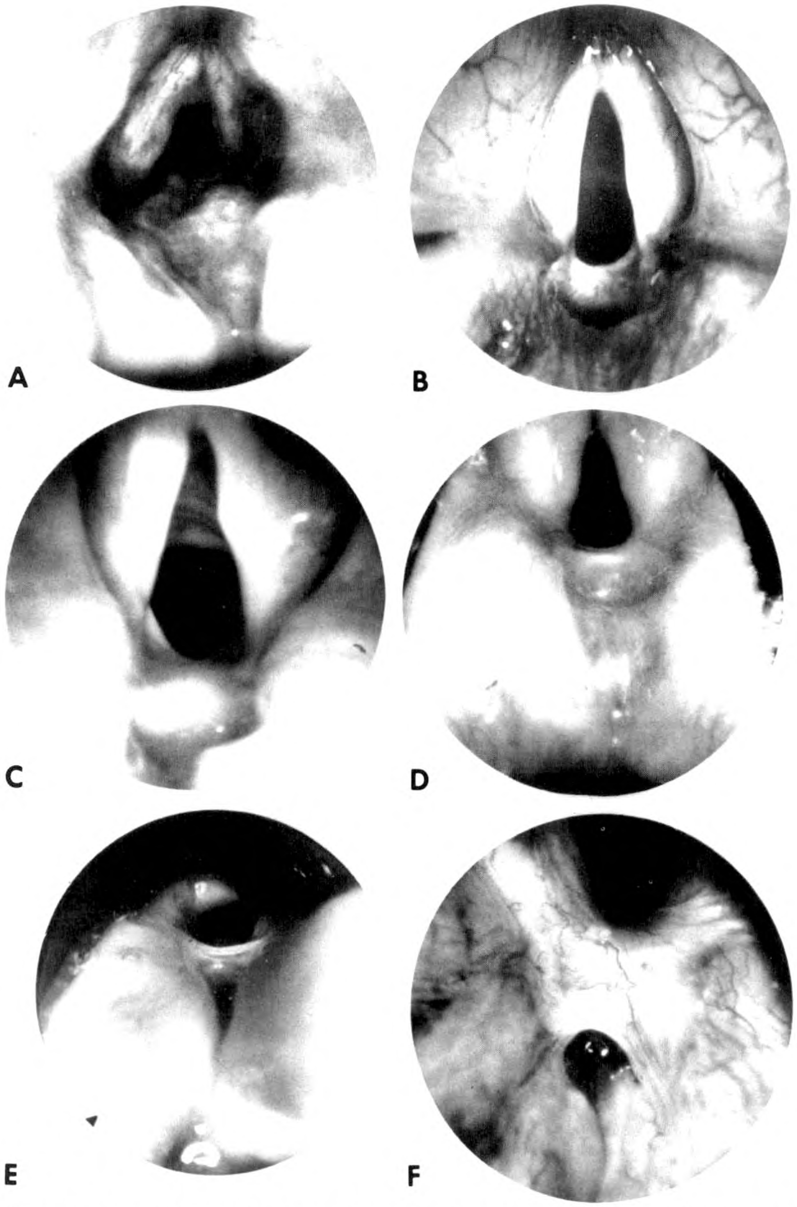

Fig 7. Formation of posterior glottic stenosis. A) Several weeks after prolonged intubation, proliferative granulation tissue in posterior glottis is beginning to mature to scar tissue. There is some clockwise rotation of glottis. B) Mature posterior glottic stenosis with crescentic anterior edge. C) Posterior glottic stenosis extending into subglottis. Some irregular scarring of right vocal cord. D) Mature posterior glottic stenosis seen with 30° telescope; small depression is often present just behind anterior edge of stenosis. E) Thin posterior glottic stenosis stretched by forcibly abducting arytenoids with tip of laryngoscope. F) Close-up of firm, fibrous posterior glottic stenosis. Posterior opening is blind. Cricoarytenoid joints are fixed.

fibrous nodule is unlikely to improve the voice.

Other distinct, well-known entities include the following.

Intubation Granuloma. A typical localized, rounded intubation granuloma is a yellow-red pedunculated mass arising from the vocal process and nearby medial surface of the arytenoid (Fig 5). The patient has dysphonia, a feeling of "something there," and large or bilateral granulomas can cause increasing airway obstruction.

Interarytenoid Adhesion. This transverse fibrous bridge forms when granulation tissue or ulcerated surfaces fall together and heal across the midline, leaving a triangular anterior opening and a smaller, posterior, rounded opening (Fig 6) so that the vocal cords are tethered to one another, causing partial airway obstruction. The anterior opening is often mistaken for the glottis, and an erroneous diagnosis of bilateral vocal cord paralysis is made. Simple division with the laser or small microsurgery scissors should give a satisfactory outcome.

Posterior Glottic Stenosis. Transverse scar tissue between the arytenoids at the glottic level, often extending downward into the posterior subglottic region (Fig 7), is a common and major cause of morbidity after prolonged intubation in both adults and children. During intubation, acute annular ulceration in the posterior glottic space results in proliferation of persistent granulation tissue after extubation; maturation to scar tissue over many weeks produces posterior glottic stenosis. In severe cases the posterior intercartilaginous parts of the vocal cords are tethered together. Partial or even total fixation of the

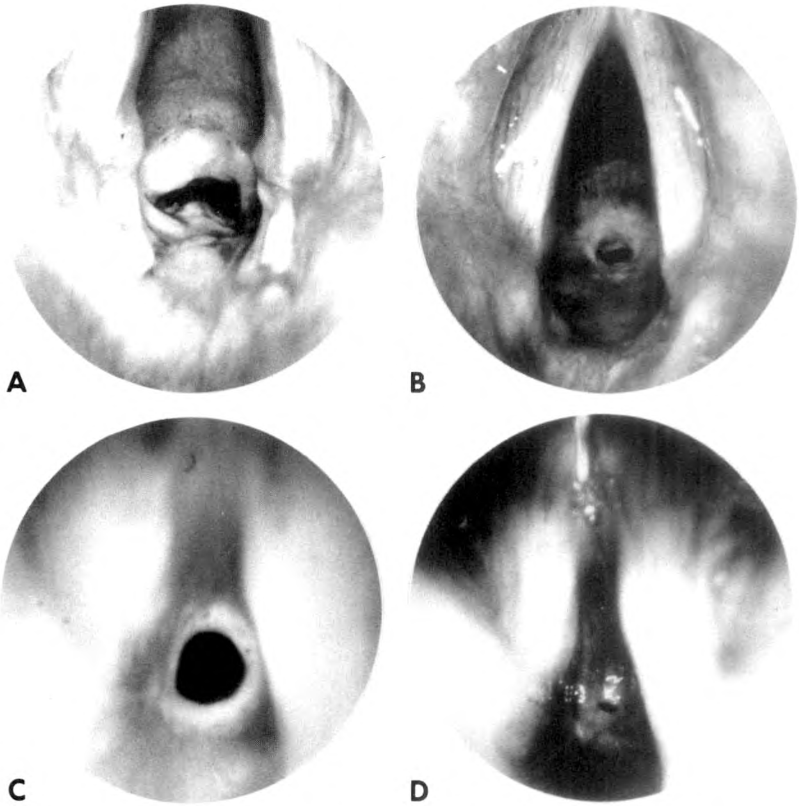

Fig 8. Subglottic stenoses. A) Acute, circular, symmetric ulceration in subglottic region within cricoid in adult, after removal of endotracheal tube during prolonged intubation. B) Same patient 2 months later. Mature subglottic stenosis with residual lumen of only 1 or 2 mm. C) Subglottic stenosis not far below glottic level in premature infant intubated and ventilated for respiratory distress syndrome. D) Mature subglottic stenosis with tiny pinhole lumen in infant.

cricoarytenoid joints with the vocal cords in the adducted position results in inspiratory airway obstruction, and as the voice is usually nearly normal, the condition is often misdiagnosed or poorly assessed, being confused with bilateral abductor paralysis or merely described as "laryngeal stenosis," an unfortunate and imprecise term.

In mild cases, especially those in which an intact median strip of mucosa was identified posteriorly during assessment of acute intubation damage, healing may be complete, with no functional deficit. Thorough assessment of posterior glottic stenosis is possible only at direct endoscopy with the patient under general anesthesia and use of both 0° and 30° rigid telescopes so that the transverse fibrotic scar can be identified as a web — sometimes thin and crescentic (Fig 7E), but usually firm and thick (Fig 7D) — filling and fixing the interarytenoid region. The anterior edge of the scar may be sharp or blunt, and the web may reach from the level of the interarytenoid muscle above, include the glottic level with accompanying fibrous ankylosis of the cricoarytenoid joints, and extend below into the subglottic region. Fixed or partly fixed joints can be diagnosed confidently only by direct palpation under general

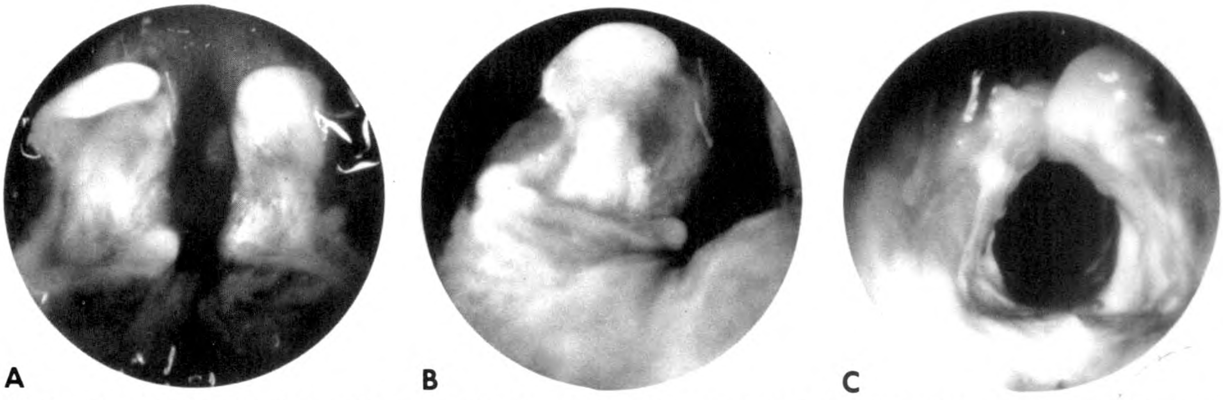

Fig 9. Acute, deep ulceration in posterior larynx. A) Close-up view of bilateral ulcerated troughs with intact median strip of mucosa posteriorly. Later formation of posterior glottic stenosis is not likely. B) Closer view using 30° telescope to show ulcerated trough on left side. Line of cricoarytenoid joint can just be seen. C) Annular ulceration involving circumference of posterior glottis with granulation tissue at vocal processes. Later formation of posterior glottic stenosis is likely.

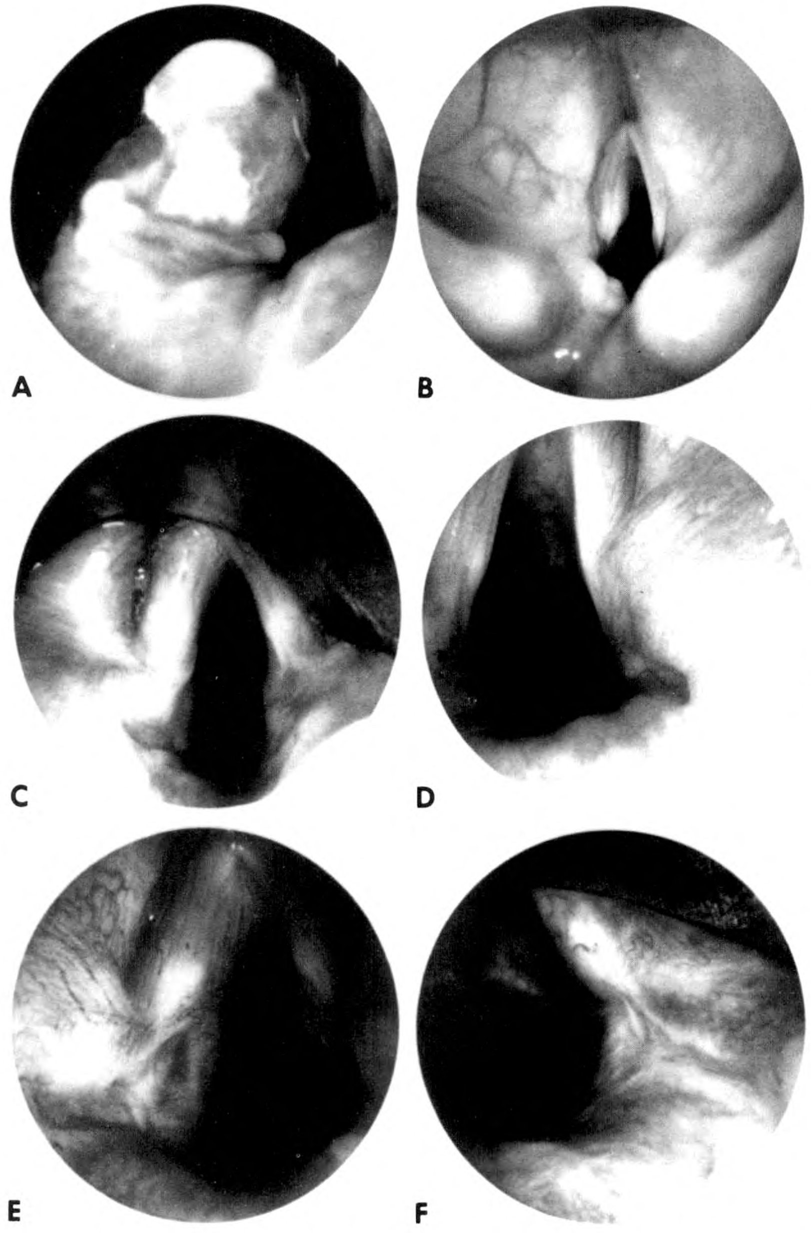

Fig 10. Formation of healed furrow. A) Acute ulcerated trough, same photograph as in Fig 9B. After extubation, ulcerated trough resolves and scar tissue forms healed furrow. B) Typical appearance of healed furrow with 0° telescope from above. There appears to be deficit posteriorly in left vocal cord, but it is healed furrow. C) Healed furrow seen at close inspection on left side. D) Healed furrow on right side. E) Healed furrow on left, showing its relationship to cricoarytenoid joint. F) Close-up of right healed furrow in same patient as in E.

anesthesia, but lesser degrees of joint dysfunction can merely be suspected because of surrounding scar tissue. Treatment directed only at coexistent, more easily recognized subglottic stenosis may not be effective when posterior glottic stenosis remains unidentified and untreated.

Subglottic Stenosis. Subglottic stenosis (Fig 8) is present when the subglottic diameter (below the level of the glottic opening and above the level of the inferior margin of the cricoid cartilage) becomes sufficiently narrow to cause symptoms of airway obstruction. In adults most cases of subglottic stenosis are caused by prolonged intubation.

In the pediatric age group obstruction from prolonged intubation is more likely where there is a preexisting congenital subglottic stenosis[25] due to an abnormality of the cricoid cartilage or thickening of the subglottic soft tissues. In a healthy, full-term newborn the diameter of the normal subglottic region is approximately 4.5 mm, and infants with a cartilaginous congenital subglottic stenosis may become symptomatic only after endotracheal intubation causes edema, granulation tissue, or, later, fibrosis.

The tissues inside a normal cartilaginous cricoid ring are susceptible to mechanical trauma that might be caused by prolonged intubation provoking edematous swelling of the loose submucosal connective tissue and critical airway obstruction — a sequence of events seen sometimes in the critically ill premature neonate ventilated for respiratory distress syndrome. The cricoid cartilage permits swelling only into the lumen, at the expense of the airway.

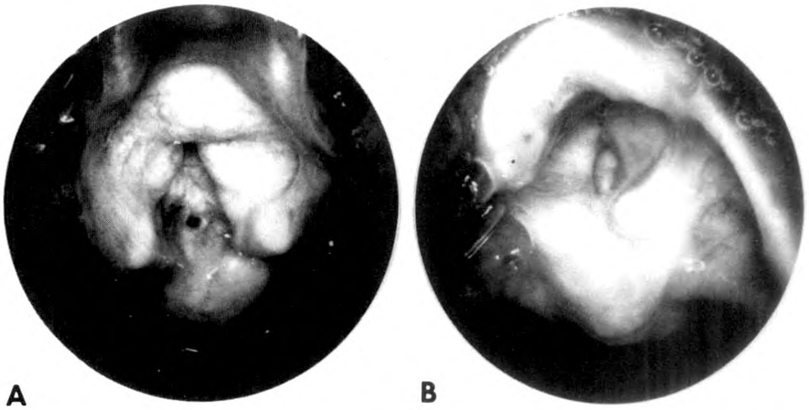

Fig 11. Glottic obstruction. A) Almost total obstruction in glottic area. B) Total glottic obstruction following prolonged intubation and made worse by repeated laser treatments.

A so-called "hard" subglottic stenosis can be caused by a congenital abnormality of the cricoid cartilage or by firm fibrotic scar tissue. A so-called "soft" subglottic stenosis can be caused by granulation tissue, submucosal fibrosis, submucosal hyperplasia and dilation of seromucinous glands, ductal retention cysts, or a combination of these factors.[26]

At endoscopy the appearance of each subglottic stenosis, whether in adults or children, is different (Fig 8). There may be a thin, membranous, diaphragm-like web or firm fibrotic scar tissue disposed circumferentially or in some irregular manner. In some patients there is not only subglottic stenosis but some other damage due to prolonged intubation — for example, posterior glottic stenosis or some fixation of the cricoarytenoid joint. Subglottic stenosis must be confirmed and evaluated at endoscopy, and a lateral airway x-ray film is essential in appraising the thickness of the stenosis.

Complete Obstruction. Near-total or total obliteration of the lumen (Fig 11) sometimes occurs at the glottic and subglottic level in advanced cases. Iatrogenic trauma from ill-judged repeated attempts at dilation or from excessive laser surgery can worsen the damage initially caused by prolonged intubation.

Ductal Retention Cysts. Submucosal ductal retention cysts (Fig 4E), large or small, develop in the subglottic region, usually posteriorly, in infants who have undergone prolonged intubation.[27] The cysts are due to irritation and obstruction of the duct of a mucus-producing gland. Large cysts can cause airway obstruction and usually present months after extubation. The diagnosis may be suggested by detecting smooth, asymmetric, subglottic lateral or posterior masses on radiography, but must be confirmed at laryngoscopy. Small cysts, single or multiple, are quite often found incidentally as rounded or flattish cysts a few millimeters in diameter in the subglottic or upper tracheal region.

Vocal Cord Paralysis. Unilateral and, rarely, bilateral vocal cord paralysis[28] may be a complication of either short-term or prolonged intubation. Branches of the recurrent laryngeal nerve are thought to be compressed between the arytenoid and the laryngeal cartilages. Spontaneous recovery can usually be expected within 6 months.

Dislocation of Arytenoid. Trauma to the arytenoid region sometimes follows blind intubation or use of an introducer in the endotracheal tube; dislocation of the arytenoid[23] may be the result, more commonly on the left side (Fig 4C), since intubation is through the right side of the mouth, with the tube tending to go toward the left side of the larynx. The patient complains of persistent hoarseness and pain on swallowing; indirect laryngoscopy shows a displaced arytenoid and limitation of movement. The problem is seldom recognized in the acute stage, and attempts to manipulate the cartilage into position are unlikely to be successful. It seems that most cases present later with limited movement or fixation in the joint.

PRINCIPLES OF TREATMENT

Management of the consequences of prolonged intubation depends on an understanding of the pathogenesis of the different acute and chronic injuries, and the complex interrelationships when there is more than one problem — for example, subglottic stenosis and intubation granuloma (Fig 4G). Endoscopic evaluation of intubation trauma is necessary for final assessment of the site, nature, and degree of the injuries.

The principles of treatment will be briefly outlined, first for acute injuries and second for chronic injuries.

Acute Injuries During Intubation. Included here are minor injuries such as edema in the vocal cords, surface mucosal ulceration, generalized inflammation, and early granulation tissue at the vocal processes or in the posterior larynx that do not cause lasting changes, and when the endotracheal tube is

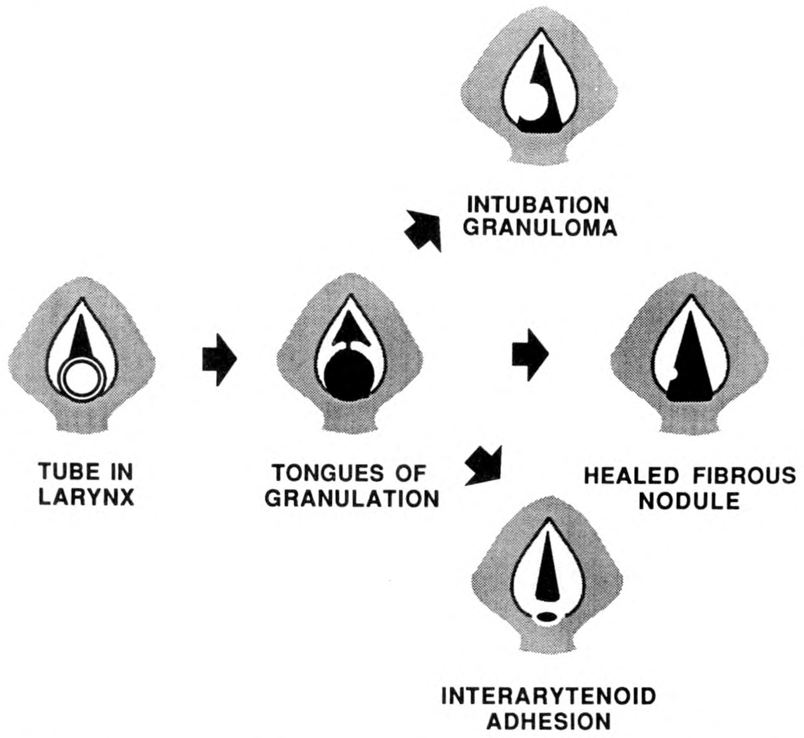

Fig 12. Flow chart showing consequences of granulation tissue forming during prolonged intubation and three distinct chronic changes that can occur: intubation granuloma, healed fibrous nodule, and interarytenoid adhesion.

removed they are likely to resolve, with return of normal laryngeal function.

Severe injuries with deep ulceration into the cartilage of the arytenoid, into the cricoid, or into the cricoarytenoid joint or ulceration involving all of the posterior glottic space indicate the need either for extubation as soon as possible or for tracheotomy and removal of the endotracheal tube, with appropriate precautions including antibiotic treatment to minimize infection.[13] The likelihood of symptomatic chronic intubation changes becoming manifest weeks or months after extubation requires ongoing assessment.

If dislocation of the arytenoid joint is detected during the acute phase, an attempt should be made to reposition it, although there is little evidence that this can be effectively achieved.

Other injuries are seen, but there is little that can be done to "treat" acute intubation damage that causes moderate granulation tissue or ulceration. A judgment might be made in selected cases to remove tongues of granulation tissue on one or possibly both sides in an attempt to assist in successful extubation. However, it has not been shown that removal of obstructing granulation tissue is necessarily beneficial. Theoretically, removal might promote troublesome bleeding. Certainly, if granulation tissue is removed bilaterally, as the tissues swell after extubation there is a possibility that the traumatized surfaces could adhere and form an interarytenoid adhesion.

Chronic Injuries After Extubation. The chronic changes caused by granulation tissue (Fig 12) and ulceration (Fig 13) can be shown diagrammatically in flow charts. Interarytenoid adhesion detected in the first few days or weeks after extubation, before it becomes scar tissue, can be gently broken down or divided, but a further examination should be done within 7 to 10 days to check for recurrence. A mature, firm interarytenoid adhesion can be divided at microlaryngoscopy with the laser or microsurgery scissors. Although subsequent examination sometimes shows small, rounded healed areas on each side, in the absence of other changes, the voice is usually satisfactory.

Posterior glottic stenosis is another form of chronic injury. Scar formation in the posterior larynx is a relatively common cause of major morbidity after prolonged intubation. Mild, uncomplicated lesions may not be detected, and others may not warrant treatment, but patients with airway obstruction need relief.

A simple endoscopic treatment that may alleviate symptoms in some cases involves vertical division of the posterior scar tissue with either a laser or, more effectively, a curved No. 11 scalpel blade on a long handle. The web is boldly divided in the midline from the subglottic region to the interarytenoid region, deep enough to feel the tip of the scalpel blade on the cricoid lamina. The tissues "spring" apart, leaving a deep V-shape gap in the scar tissue, the stenosis is "released," and the patient may have partial but

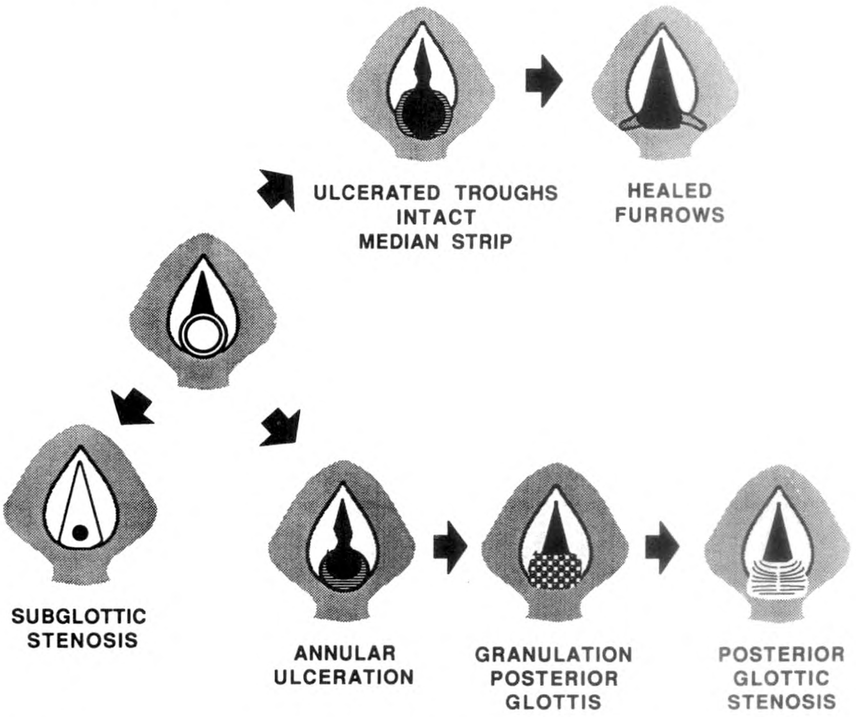

Fig 13. Flow chart showing consequences of ulceration following prolonged intubation: healed furrows, posterior glottic stenosis, and subglottic stenosis.

dramatic relief of airway obstruction. Subsequent restenosis is likely and may require repeated division, sometimes every 6 months and sometimes every few years; when this conservative approach is unsuccessful, other forms of surgical treatment are required.

In severe cases laryngofissure allows the choice of one or more procedures: excision of the weblike scar for placement of a free or pedicled mucosal graft to cover the denuded area, arytenoidectomy for joint fixation, vocal cord lateralization, or a cartilage graft to widen the posterior cricoid lamina. In long-standing cases with extensive scarring, fibrosis, and fixation of both cricoarytenoid joints, a lasting beneficial result is difficult to achieve.

Subglottic stenosis is also a form of chronic injury. The anterior cricoid split[29] with or without immediate cartilage graft[30] may allow extubation of infants with subglottic stenosis. Expansion of the cricoid cartilage is achieved by an anterior midline incision to split the cartilage of the cricoid, the first and second tracheal arches, and the lower third of the thyroid cartilage.

In older patients thin, weblike subglottic stenotic tissue can usually be successfully treated with endoscopic application of the carbon dioxide laser. Laser treatment, however, is inadequate for extensive scar tissue greater than 1 cm in vertical dimension in adults; these cases require open augmentation laryngoplasty using anterior and occasionally posterior grafts of cartilage or bone to widen the cricoid cartilage and enlarge the subglottic airway sufficiently to permit decannulation.[31,32] There is no agreement about which of the many surgical approaches gives the best results; the surgeon needs to be versatile, using the most suitable procedure for each problem, after careful preoperative evaluation. Augmentation surgery is seldom indicated when a tracheotomy has not been necessary. The grading of subglottic stenosis by percentage of laryngeal lumen obstruction, introduced by Zalzal and Cotton,[32] is useful in recording, quantifying, and evaluating the results of treatment.

When stenosis of the subglottic region also involves the glottis or is so severe that there is total obliteration, reconstructive laryngoplasty is difficult, having approximately a 50% success rate; if it is unsuccessful, the patient will have a permanent tracheotomy.

For difficult cases or failed graft operations, surgical resection of the anterior arch of the cricoid and part of the posterior cricoid lamina below the cricothyroid articulation has been successful, but great care must be exercised to minimize injury to the recurrent laryngeal nerves.

Dilation of a firm, mature laryngeal stenosis, whether posterior glottic or subglottic, is not recommended, as repeated forceful tearing of scar tissue may promote even more fibrosis.

Attempts at treatment of subglottic stenosis by inappropriate, overenthusiastic, and unwarranted repeated laser surgery can create more fibrosis, aggravating the original stenosis so that surgical reconstruction is more difficult.

Ductal retention cysts may need no treatment if they are small and insignificant, but for multiple larger cysts, which may cause airway obstruction, use of the laser is the treatment of choice. At microlaryngoscopy, as the roof of the cyst or cysts is vaporized, mucus exudes from them. There is seldom recurrence.

A fixed or dislocated arytenoid[33] causing serious airway obstruction should be evaluated for arytenoidectomy, whether endoscopic or through a laryngofissure.

Tube Removal. When removal of the endotracheal tube has already been unsuccessful in the ICU or when removal is to be attempted in the presence of moderate laryngeal edema, granulation tissue, and some ulceration, additional steps can be taken. Although the use of steroids is controversial and not proven to be beneficial, they are used regularly by many ICU physicians and laryngologists. A smaller endotracheal tube can be placed for 24 to 48 hours. In addition, extubation is facilitated in very low birth weight neonates intubated and ventilated for respiratory distress syndrome who cannot otherwise maintain independent ventilation after extubation, if the airway is supported by nasal continuous positive airway pressure with a mask or with nasal prongs.[34,35] This technique may also decrease the frequency and severity of apnea and bradycardia, which often contribute to respiratory failure.

PREVENTION

In most cases prolonged intubation for 5 to 7 days, or occasionally longer, produces no permanent long-term sequelae; the injuries are readily reversible once the tube is removed. On the other hand, in a small number of patients intubation for only a few days can cause advanced changes, and there are certain "at-risk" situations. Some of the worst laryngeal damage has been found to occur as follows.

1. In unconscious patients with a head injury who are allowed to remain intubated for a long time.
2. In children cared for in an adult hospital where a large, cuffed, or even a rubber tube has been used.
3. When prolonged intubation is followed by a tracheotomy in a patient who remains unconscious.
4. In patients with multiple systemic problems who have poor cardiac output or other major organ failure.
5. In infants with preexisting congenital subglottic stenosis.
6. After prolonged or repeated intubation for ventilation of very low birth weight premature infants.
7. In pediatric patients intubated for airway obstruction due to acute laryngotracheitis.[10]
8. More often in an abnormal larynx than in a normal larynx.

Therefore, when some of the factors important in morbidity from intubation are present, special precautions should be taken. The outside diameter of the tube is most important. A tube of smaller diameter can usually be relatively safely used, or one of the specially constructed tubes such as the Lindholm tube[11] or the prototype tube suggested by Weymuller[5] should be considered. Gastroesophageal reflux should be vigorously treated and static secretions around the endotracheal tube reduced. Infection from a tracheotomy performed for laryngeal intubation trauma must be minimized by local and systemic therapy, as stomal contamination may prolong healing and predispose to scar formation.

The ideal endotracheal tube for prolonged intubation would be inexpensive, be made of synthetic material with a smooth, nonirritating surface, have no potentially toxic components, be of a low porosity, and be thermoplastic at body temperature to mold itself to body contours. It would need to be modified to disperse pressure over a large surface contact area and minimize posterior and lateral intralaryngeal surface pressures so as not to exceed capillary perfusion pressure. It would have a low-pressure, large-volume, compliant cuff.

In summary, although most intubation injuries heal leaving a normal or near-normal larynx, some patients develop airway obstruction and/or dysphonia. There may be hoarseness, tiring of the voice, change of the voice, inability to sing, or subtle changes that can be worrying, especially to professional voice users. Laryngeal stroboscopy may show a "stiff," poorly vibrating membranous vocal fold. Apparently minor changes such as an uncomplicated healed furrow, a healed fibrous nodule, or lesser degrees of cricoarytenoid joint abnormality are detectable only by careful examination, and the probability of subtle, difficult-to-document changes in the cricoarytenoid joint has been highlighted. Severe changes, such as posterior glottic stenosis or subglottic stenosis, usually require reconstructive surgery.

This paper outlines a comprehensive, practical classification of laryngeal intubation trauma and introduces new terminology to describe tongues of granulation tissue, ulcerated troughs, healed furrows, and healed fibrous nodules. Flow charts show the pathogenesis of the common long-term changes caused by granulation tissue and ulceration. Basic treatment has been briefly outlined. Awareness of the factors predisposing to laryngeal trauma and endoscopic recognition of the development of severe changes will assist in decreasing morbidity.

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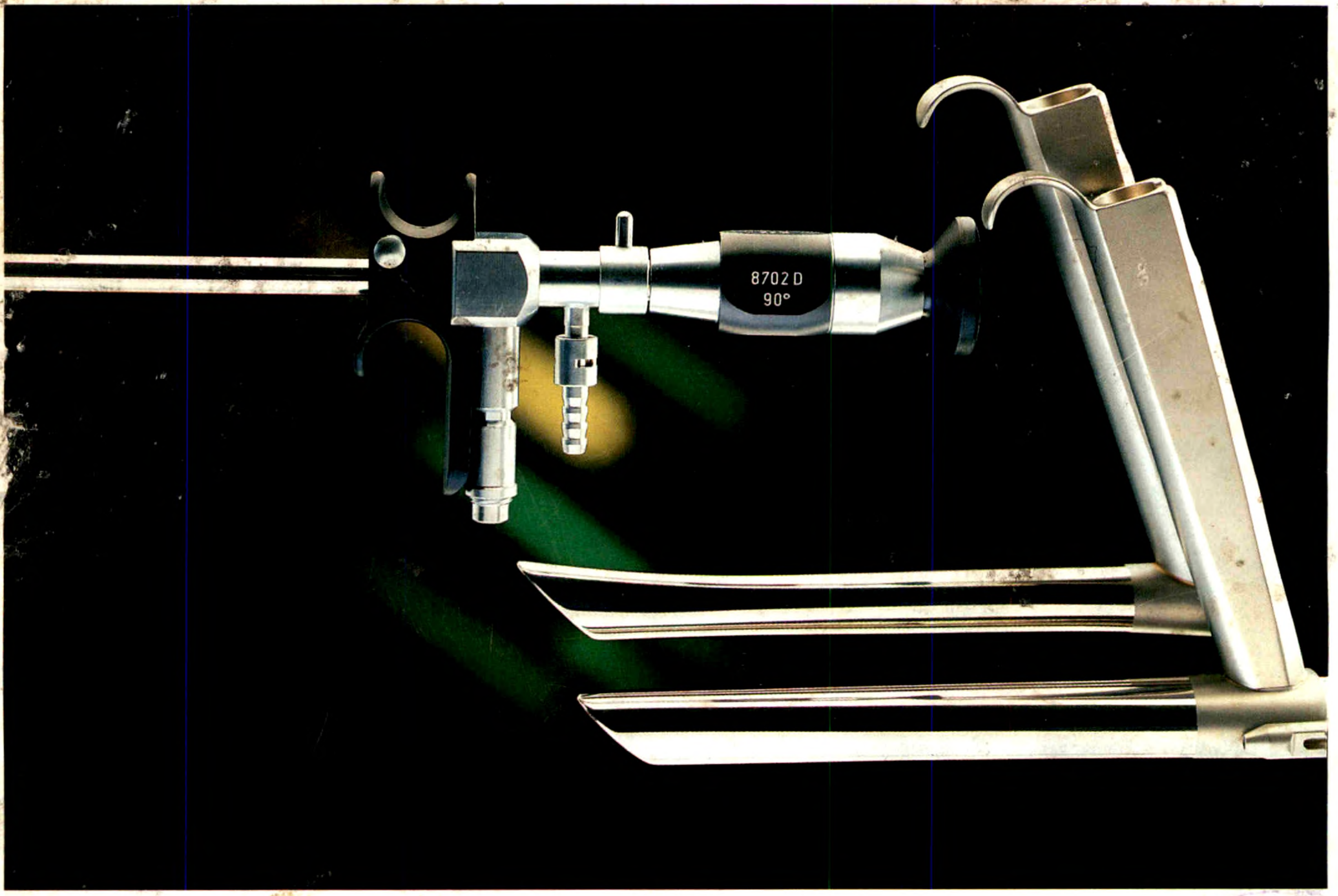

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